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RESPONSIBLE ANTIBIOTIC USE AND DIAGNOSTIC CHALLENGES IN INFECTIOUS DISEASES:

Studies in a resource-limited setting and a high-income setting

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The cover photo shows the Shri Ram Ghat on the Shipra River in Ujjain. I often returned to this place during my visits in Ujjain for inspiration and perspectives.

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Responsible antibiotic use and diagnostic challenges in infectious diseases: Studies in a resource-limited setting and a high-income setting

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By

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To my grandmother, Barbro

PREFACE

During my time at medical school, some of my classmates and I had the opportunity to participate in an elective course in Bhubaneshwar, India. We were to study diabetes in a hospital for three weeks, just before Christmas. We were all very eager to experience the health care, food and culture. I was up every morning at sunrise for a morning walk. I watched the 'wallahs' selling chai from sizzling pots, the kids washing and playing outside their houses, the women sweeping the streets, and smelled the garbage burning in small piles along the road. Many of us longed for home; we ordered pizza to get a break from all the curries; we took turns in the bathroom with the feared "Delhi belly", and the coffee and "kokosbollar" that our teacher brought from Sweden were finished after a few days. This was my first trip to India, but little did I know that it would be the first of many to the incredible country that has its own special place in my heart.

A few months after we came home, it was time to plan for the degree project that was to include an entire semester. I had no experience of research, and all I knew was that I wanted to go back to India. I started to go through the professors on the Karolinska Institutet's website, one by one. I put everyone who had a connection with India on a list, and finally I marked Cecilia's name with yellow highlight, as I found her research projects the most exciting. On 15 February 2013, I walked into Cecilia's office for our first meeting. There were souvenirs, fabrics and all kinds of art from around the world all over the office. She offered me a small samosa-shaped cake and said: "I have this antibiotic prescribing project in Ujjain. When would you like to go?" When I left her office, I felt that this was the beginning of a new adventure.

In the autumn of 2013, I left a rainy and dark Sweden for a couple of months in a sunny and colourful Ujjain, after being awarded a Minor Field Study scholarship from the Swedish International Development Cooperation Agency (Sida). Megha, my co-supervisor, welcomed me with open arms and guided me through the college and the hospitals, introduced me to the research project and taught me how to eat curries with roti, using only my right hand. From early morning to late afternoon, six days a week, I sat opposite Megha at the large desk in her office, where I analysed data, interpreted results and wrote my first manuscript. I also had the opportunity to visit the two hospitals where the data were collected, and to meet some of the clinicians, which was very interesting. In the evenings I walked the streets of Ujjain, taking photographs, sipping burning hot chai from small plastic cups, and thinking of the next analysis. I learned so much during those months in Ujjain; it was one of the most important journeys I have made for my professional and personal development. Barely a year later, I returned to Ujjain for my second project in the research group, after being admitted to the Karolinska Institutet summer research school for medical students.

In January 2016, after a three-month exchange in Oman, I graduated from Karolinska Institutet. Two weeks later, I stepped into the morning rounds at Herlev Hospital in Copenhagen to start my internship (allmäntjänstgöring). One year and numerous "rugbrødsmadder", miles by bike,

and night shifts later, I returned to Stockholm with a medical certificate. By then, it was time to apply for residency (specialisttjänstgöring). I opted for the field of clinical physiology. I have always been attracted to physiology, and it fascinated me to specialize in the area of cardiac ultrasonography, echocardiography (ECHO), where logical reasoning and advanced technology are key issues, and where I could put all my energy into exploring small details to help clinicians move forward in the management of their patients.

My work at the Department of Clinical Physiology has increased my understanding of the importance of diagnostics and its impact on the management of the patients. We diagnose, evaluate and conduct follow ups with ECHO, a method that has also been implemented in some resource-limited settings with successful results as it is less costly and often requires less personnel compared to many other imaging methods. When analysing the data from Ujjain, I was surprised that very few of the registered diagnoses were confirmed by diagnostic methods. I believe it is essential to highlight the need of diagnostic methods as a clinical tool at hospitals in such areas, where underdiagnosing of infectious diseases is common, and patients are often prescribed antibiotics on clinical suspicion of infection. The most common infectious disease that we encounter at the Department of Clinical Physiology is infective endocarditis (IE). ECHO is important for the diagnosis and management of patients with IE. When I analysed the data from the two hospitals in Ujjain, I discovered that only 9 patients out of 243,774 admitted to the hospitals between 2008 and 2017 were registered with IE. Therefore, I began to think about how to optimize the diagnostics and management of patients with IE in resource-limited settings using ECHO. Could the ECHO findings say something about bacterial aetiology in these patients, making it possible to guide the treatment of patients in settings with limited access to diagnostic methods? And so, the idea of how to complement the studies of antibiotic prescribing in Ujjain with a study of a diagnostic method; ECHO, that could improve the management of an infectious disease; IE, began to grow. I contacted Cecilia and she was positive. I talked to Maria, who was then the head of the Department of Clinical Physiology. She gave me the opportunity to conduct my doctoral studies in parallel with my clinical work and was also willing to be my co-supervisor. A detailed plan took form during the spring, and in June 2018 I was registered as a doctoral student. The day before the registration seminar, I was admitted to the Karolinska Institutet Research School for Clinicians in Epidemiology, a two-year programme including all compulsory doctoral courses. The research school formed the best possible foundation for me as a young researcher. I found great satisfaction in being able to create and understand statistical analyses, to discuss scientific arguments and be inspired by well performed and presented research. I have had the opportunity to teach medical students, and to present my research at international conferences, which has been immensely inspiring. I think research offers an endless opportunity to develop, learn and teach. When our work benefits heath care and patients, I feel that we have achieved one of the most noble outcomes.

ABSTRACT

Background: Antibiotic resistance is a globally emerging health challenge. In resource-limited settings, the burdens of infectious diseases and of antibiotic resistance are the highest. The World Health Organization (WHO) has stated that high and uncontrolled use of antibiotics in resource-limited settings contributes to the increasing prevalence of antibiotic resistance in such areas. Improvements should be based on knowledge about current antibiotic use, preventing the spread of infections, and the implementation and/or development of diagnostic methods for the improved management of infectious diseases. Limited access to diagnostic methods complicates the management of infectious diseases in resource-limited settings and increases the risk of disregarding severe infectious diseases, such as infective endocarditis (IE). Therefore, it is important to evaluate diagnostic methods that can improve the management of infectious diseases in such settings. Evaluating whether the findings of echocardiography (ECHO) could give a suggestion of the bacterial aetiology in patients with IE in a high-income setting might provide valuable information for the implementation of ECHO in resource-limited settings.

Aims: To map and describe the antibiotic prescribing practices and to highlight areas of improvement in the management of in-patients with infectious diseases in a resource-limited setting in India. Further to assess whether specific manifestations detected by ECHO were associated with certain bacterial species in patients with IE in a high-income setting in Sweden.

Methods: The studies for **Papers I-III** were conducted in a teaching hospital (TH) and a non-teaching (NTH) hospital in India. Two were prospective, cross-sectional studies (**I and II**) and one was a time series analysis (**III**). **Paper IV** was conducted in Stockholm, Sweden, as a register-based cohort study. For **Paper I**, antibiotic use was analysed at the medical intensive care units (ICUs) at the two hospitals in India. For **Paper II**, antibiotic prescribing practices were analysed with a focus on infectious diagnoses at the paediatric departments at the two hospitals in India. For **Paper III**, time series analyses over a 10-year period (2008–2017) were conducted to follow antibiotic prescribing over time among patients with severe infections at the NTH and the TH in India. For **Paper IV**, associations between IE manifestations detected by ECHO and bacterial species were assessed from a cohort of patients with IE obtained from the Swedish National Registry of Infective Endocarditis (SRIE).

Main findings: High percentages of patients at the medical ICUs were prescribed antibiotics at both hospitals (4,843 of 6,141 patients, 70%) although less than 25% of the patients were registered with an infection-associated diagnosis (**I**). At the paediatric departments, antibiotic use among patients with either acute gastroenteritis, respiratory tract infections, enteric fever, viral fever or unspecified fever was more common at the NTH (2,088 patients, 84%) compared with the TH (224 patients, 44%; $P < 0.001$). Broad-spectrum antibiotics were commonly

prescribed at both hospitals, and less than 40% of the prescribed antibiotics at both hospitals were compliant to the national list of essential medicines (37% at the TH and 24% at the NTH; $P < 0.05$) (**II**). From 2008 to 2017, the overall rate of antibiotic prescribing for patients with severe infections, as well as prescribing of first- and second-line antibiotics and so-called fixed-dose combinations (FDCs) of antibiotics increased in the NTH ($P < 0.05$). In the TH, the overall antibiotic prescribing did not change significantly, although the prescribing of second- and third-line antibiotics and FDCs of antibiotics increased during the study period ($P < 0.05$) (**III**). Among patients with IE, associations were seen between aortic valve vegetation and *Enterococcus faecalis* among patients with native aortic valves, between mitral valve vegetation and streptococci of group B or viridans group streptococci, between tricuspid valve vegetation and *Staphylococcus aureus* among patients with intravenous drug abuse, and between perivalvular abscesses as well as cardiovascular implantable electronic device (CIED)-associated IE and coagulase-negative staphylococci (all $P < 0.05$) (**IV**).

Conclusions: Antibiotic prescribing was high at the ICUs and paediatric departments at the NTH and the TH, also among patients presenting with no infection-associated diagnoses or with diagnoses for which antibiotic treatments are not recommended. Broad-spectrum antibiotics were commonly prescribed at both hospitals. From 2008 to 2017, prescribing of second-line antibiotics and FDCs of antibiotics among patients with severe infections increased at both hospitals. The findings suggest that there is a need for improved antibiotic prescribing and management of infectious diseases. A major problem in this setting as well as in similar resource-limited settings is the limited use of diagnostics, which are needed to guide the treatment and follow-up of infectious diseases. Limited use of diagnostics also increases the risk of underdiagnosing infectious diseases. Very few cases of IE were registered at the NTH and TH, which might be a result of the limited use of diagnostics. ECHO is an effective method for the diagnosis, evaluation and follow-up of patients with IE, successfully implemented in high-income settings. ECHO can be used to identify cardiac manifestations of IE but might also give a suggestion of bacterial aetiology as some IE manifestations detected by ECHO have been shown to be associated with certain bacterial species. These findings could possibly be useful also in resource-limited settings, where ECHO might improve the management of patients with IE.

LIST OF SCIENTIFIC PAPERS

I. Sharma M*, **Damlin A***, Sharma A, Stålsby Lundborg C.
Antibiotic prescribing in medical intensive care units–a comparison between two private sector hospitals in Central India.
Infect Dis (London). 2015;47(5):302–9.
*Shared first authorship.

II. Sharma M*, **Damlin A***, Pathak A, Stålsby Lundborg C.
Antibiotic prescribing among pediatric inpatients with potential infections in two private sector hospitals in Central India.
PLoS One. 2015;10(11):e0142317
*Shared first authorship.

III. **Damlin A,** Sharma M, Marrone G, Stålsby Lundborg C.
Antibiotic prescribing among patients with severe infectious diseases in two private sector hospitals in Central India – A time series analysis over 10 years.
BMC Infect Dis. 2020;20(1):340.

IV. **Damlin A**, Westling K, Maret E, Stålsby Lundborg C, Caidahl K, Eriksson MJ.
Associations between echocardiographic manifestations and bacterial species in patients with infective endocarditis: a cohort study.
BMC Infect Dis. 2019;19(1):1052.

These papers are referred to in the text by their Roman numerals (I–IV).

CONTENTS

LIST OF ABBREVIATIONS

ATC	Anatomical therapeutic chemical
CI	Confidence interval
CIED	Cardiovascular implantable electronic device
CoNS	Coagulase-negative staphylococci
DDDs	Defined Daily Doses
ECHO	Echocardiography
ESBL	Extended-spectrum beta-lactamase
FDC	Fixed-dose combination
HACEK	Combination of *Haemophilus* species, *Aggregatibacter* species, *Cardiobacterium hominis, Eikenella corrodens* and *Kingella Kingae*
IAP-LEM	Indian Academy of Paediatrics List of Essential Medicines
ICU	Intensive care unit
IE	Infective endocarditis
IV	Intravenous
KUH	Karolinska University Hospital
LMICs	Low- and middle-income countries
NLEMI	National List of Essential Medicines in India
NTH	Non-teaching hospital
OR	Odds ratio
PCR	Polymerase chain reaction
RHD	Rheumatic heart disease
RTI	Respiratory tract infection
SRIE	Swedish National Registry of Infective Endocarditis
TEE	Transoesophageal echocardiography
TH	Teaching hospital
TTE	Transthoracic echocardiography
WHO	World Health Organization
WHOCC	World Health Organization Collaborating Centre for Drug Statistics Methodology
WHOLEM	World Health Organization List of Essential Medicines

1 INTRODUCTION

1.1 ANTIBIOTIC RESISTANCE

1.1.1 Antibiotic resistance from a global perspective

Already in his Nobel Prize speech in 1945, the father of penicillin, Alexander Fleming, warned that bacteria could become resistant to antibiotics (1, 2). Since then, antibiotic resistance has developed into a global problem that continues to increase every year (1). Antibiotic resistance emerges when bacteria change their response to antibiotics, so they are not harmed or destroyed (3). To be precise, mutations or vertical and horizontal gene transfer occur in the bacteria, and selection pressure from antibiotic use contributes to the development of strains of bacteria that are less harmed by antibiotic treatment (4). Humans or animals that become infected by antibiotic-resistant bacteria might suffer because antibiotic treatments will be less effective or totally dysfunctional. The treatment alternatives for the infection caused by resistant bacteria will be reduced, or in the worst case scenario there will be no effective treatment available for such infections (5). Antibiotic resistance can occur because of extensive and/or inappropriate use of antibiotics, as the bacteria in the infected person progressively evolve decreased susceptibility (4). Infections with antibiotic-resistant bacteria can also occur if a person is infected by resistant bacteria or if bacteria in the human microbiome are resistant to antibiotics and transfer their resistance to other pathogens (4). Antibiotic resistance can affect anyone, regardless of age, sex and ethnicity. It is known that poor hygiene increases the risk that resistant bacteria will spread among populations, which is a major problem in resource-limited areas with poorly controlled hygiene practices (6). As more bacterial strains develop resistance, more classes of antibiotics will become less effective. If we do not address the problem, simple infections will eventually be life-threatening and cause unnecessary deaths (1, 7, 8). Thus, many of our common health care procedures worldwide such as surgery, organ transplantation and cancer therapy can be complicated because of antibiotic resistance, as effective antibiotic treatment is essential for the safety of these procedures (4, 9).

In 2015, the World Health Organization (WHO) adopted a global action plan on antimicrobial resistance, aiming to ensure the long-term, successful prevention and treatment of infectious diseases and to keep antibiotics as an available, effective and safe treatment with quality-assured medicines, prescribed in a rational way (10). A further aim for the Member States was to prepare national, multi-sectoral action plans that should be implemented through education about good prescribing, information on how antibiotic resistance arises and spreads, and by highlighting the need for sustainable investment through research and development (10). During 2018–2019, 117 Member States had prepared national action plans, 36 were in the planning phase, but six had no national action plan (11).

1.1.2 Antibiotic resistance in resource-limited settings

Measures to contain and slow down the emergence of antibiotic resistance are particularly important in resource-limited settings where the burdens of infectious diseases and of antibiotic

resistance are the greatest (4, 12, 13). A major challenge in resource-limited settings is to assure the balance between improved antibiotic access for appropriate therapeutic uses and to restrict inappropriate access to antibiotics (13, 14). The emergence and spread of antibiotic resistance are worse where antibiotics can be purchased without a prescription, which is common in low- and middle-income countries (LMICs) (4, 6, 15-17). Another problem involves environmental factors that contribute to the dissemination of antibiotic resistance in LMICs, such as drug manufacturing, hospital waste, and antibiotic overuse in food animals (14). As LMICs often have fewer public health protective measures compared with high-income countries, people in these areas are often more exposed to environmental or human reservoirs of antibiotic-resistant bacteria (14). To minimize the emergence of antibiotic resistance that arises from the interface between humans, animals and the environmental factors, the so-called "One Health" approach is recommended (14). This aims for better public health outcomes by planning and implementing programmes, policies, legislation and research, through multidisciplinary collaborations (10, 14). The WHO, the World Organization for Animal Health, and the Food and Agriculture Organization of the United Nations are examples of organizations that are actively working with LMICs to develop partnerships, antibiotic stewardship programmes and strategic groups, to contain the emergence of antibiotic resistance (10, 14, 16).

1.2 ANTIBIOTIC USE

Antibiotics are used after being prescribed or sometimes after their purchase as over-the-counter medicines for the treatment of, or prevention of, infectious diseases in human health care. Antibiotics are also used in animal production and in agriculture. The use of antibiotics needs to be improved globally to avoid the further development of antibiotic resistance. There is a need for improvements in antibiotic prescribing practices and also in the prevention of the spread of infections to reduce the need for antibiotic treatments (5). The increasing prevalence of antibiotic resistance contributes to high costs, as the need for a greater number of treatments and more extensive treatments increases, hospital stays are extended, and workplace production decreases as people become ill and even die from infections that cannot be treated (12). To reduce and rationalize the use of antibiotics, there is a need for spreading knowledge about how antibiotic resistance occurs, how infections can be prevented and how to map resistance patterns and the use of antibiotics by continuous research (16, 18). Further, investments in new medicines, vaccinations, information on good hygiene and sanitation routines and implementation and/or development of diagnostic methods for infectious diseases are required (12). It is also important to control the use of antibiotics outside the human sector, for instance in animal husbandry and agriculture and in the food industry as these are significant areas of antibiotic overuse (7). All improvements and developments in improving actions are important and are required in all countries every day.

This thesis focuses on the prescribing of antibiotics for, and management of, infectious diseases among in-patients: i.e., patients who are hospitalized while they receive their treatment.

1.2.1 Rational use of antibiotics

When antibiotics are to be prescribed, it is important that this is done correctly to avoid the development of antibiotic resistance (19). Antibiotics should only be used for relevant indications, with the correct choice, in the correct dosage, for the correct treatment duration and by a suitable route of administration (20). The aim should be targeted treatment: i.e., treatment with an antibiotic that is effective against the pathogen causing the infection (21-23).

Antibiotics are often prescribed empirically for in-patients when infectious aetiology is suspected, i.e., without knowledge of which bacteria caused the potential infection. Empirical antibiotic treatment should be chosen for the most likely pathogen causing the infection and site of infection, adjusted for local susceptibility patterns, and if necessary, reconsidered after daily examination and clinical evaluation of the patient (18, 24). Ideally, empirical treatment should be replaced with a targeted treatment regimen after results from diagnostics such as pathogen cultures and susceptibility tests (25). The WHO recommends empirical antibiotic treatment only if certain severe infectious diseases are suspected: epiglottitis, pneumonia, peritonitis, pyelonephritis, cellulitis, erysipelas, septic arthritis, infective endocarditis (IE), meningitis and sepsis (25). There are detailed international guidelines for empirical antibiotic prescribing when any of these diagnoses are suspected (23, 25, 26). However, in resource-limited settings with reduced or absent access to diagnostic methods, most of the antibiotic prescriptions are empirical, based on a clinical suspicion of infection. In such settings, treatments with so-called broad-spectrum antibiotics are commonly prescribed, namely antibiotics that cover a larger number of bacteria than the narrower-spectrum, more targeted alternatives, also if these are not recommended for the suspected diagnosis (4, 16, 27, 28). Broad-spectrum antibiotics are not always more effective than more targeted, narrower-spectrum types (29, 30). Unnecessary use of broad-spectrum antibiotics might contribute to the development of resistant bacteria or to adverse events such as toxicities and hypersensitivity reactions (31, 32).

One way to improve the use of antibiotics and reduce the development of antibiotic resistance is to develop and implement guidelines for antibiotic prescribing (33). Jaggi et al. showed a significant decrease in carbapenem-resistant Pseudomonas after implementation of an antibiotic prescribing policy at an Indian hospital (34). Subsequently, further improvement was made when an antimicrobial stewardship committee was developed and implemented at the hospital, which resulted in a decrease of carbapenem-resistant Pseudomonas (34). In 2014, Chandy et al. reported on the distribution of brochures with revised guidelines for antibiotic prescribing at a hospital in India. The brochures could also be accessed through the hospital's intranet. After these arrangements, the monthly use of antibiotics decreased by 37% (35).

It is very important to have access to prescribing guidelines that are adapted to the latest reports of resistance and susceptibility in order to make good decisions for both empirical and targeted antibiotic prescribing (16, 23, 24). On a national level, there is a need for lists of essential medicines and guidelines for the treatment of community-acquired and health-care-associated infections, which can provide a base for local prescribing guidelines and education at hospitals

and local health centres. This could contribute to reducing the unnecessary prescribing of broad-spectrum antibiotics, limit the development of antibiotic resistance, improve public health, improve patients' health outcomes, avoid adverse events and possibly reduce the pressure on health-care services (16). The WHO produces many lists of essential medicines, including antibiotics, which can be used as a guide for the development of local antibiotic prescribing guidelines (36). The guidelines should be adapted for available resources and developed for targeted interventions to implement then at a local level (37).

1.2.2 Antibiotic use in India

Historically, access to antibiotic treatment has been limited or absent in many LMICs, including India (15, 38). However, recent studies of antibiotic use in India have shown that the consumption of antibiotics is high and increasing, both in private and public health care, since the access to antibiotics has increased (33). There are associations between high domestic production of antibiotics, rising incomes, lower costs of antibiotics, lack of control of antibiotic use in health care, informal health-care providers, over-the-counter sales of antibiotics and rising consumption of antibiotics in LMICs, especially in India (38-41). Informal health-care providers–those without a formal degree in medicine who are not registered as health-care practitioners–are common in resource-limited settings where the health-care systems are weak (42). In rural India, these informal health-care providers, which include unqualified doctors, spiritual healers, unqualified medicine vendors and traditional birth attendants, constitute more than 50% of all active health-care providers (42-45). Khare et al. collected prescriptions from 12 informal health-care providers in rural India over 18 months and found that 74% of the prescriptions included antibiotics and 95% of antibiotics prescribed were of broad-spectrum types, indicating that there is a need for interventions to reduce unnecessary prescribing of antibiotics among such informal health-care providers (39). Supporting this, Mohanta et al. showed that there are extensive needs regarding the improvement of private sector health care in India, where uncontrolled antibiotic prescribing is common, and antibiotics can often be bought over the counter without prescriptions (46). It has been stated that the consumption of broad-spectrum antibiotics is common in India and that there are needs for improvements to rationalize antibiotic prescribing practices (47). Further, fixed-dose combinations (FDCs) of antibiotics are commonly prescribed in India, although not recommended because FDCs have been shown to drive antibiotic resistance, contributing to further unnecessary use (48). Antibiotic dosage should be tailored for the individual patient, which is often not possible while prescribing FDCs. Some FDCs, including unapproved formulations, are known to be used widely in India (49-52). In 2016, the Indian Government banned approximately 330 FDCs, of which 19% included antibiotics (53). Nevertheless, there are more than 118 FDCs of antibiotics available on the Indian market (53).

To analyse the prescribing of antibiotics can be difficult, depending on how the health-care system is structured. In India, patient records might not be computerized, so data collection and mapping of antibiotic use can be difficult (22). It has also been shown that there are difficulties in mapping resistance and susceptibility patterns because in many areas, samples are not sent

for culture on a regular basis (22, 33). To be able to use effective and sustainable treatments now and in the future, there is a need to focus on actual antibiotic prescribing practices and on the development and implementation of diagnostic methods for better management of infectious diseases in resource-limited settings.

1.3 INFECTIOUS DISEASES

Previous studies from India have shown that critically ill patients, such as patients in intensive care units (ICUs) and patients with a high suspicion of infectious causes of illness–for instance children–are often prescribed antibiotics although they might not have a relevant indication for such treatment (16, 28, 54). According to guidelines, antibiotics should only be prescribed to patients with an infection, or in certain cases for the prevention of one (16, 23, 24). These guidelines are often based on the site of infection, and/or which bacteria have caused the infection, and their susceptibility patterns. This can be difficult in settings with limited access to diagnostic methods, where the diagnoses for which the patients are prescribed antibiotics are often based on clinical suspicion rather than being verified by diagnostic procedures (4, 22, 35). Symptomatic management of a suspected infection using antibiotic treatment can be effective, but can also result in overuse and potential risks for the development of antibiotic resistance (55, 56). The lack of access to diagnostic methods in resource-limited settings is a major obstacle to combatting infectious diseases (57). When access to diagnostic methods is lacking, the risk of disregarding important infections that require rapid and extensive antibiotic treatment increases (58). Life-threatening infections such as sepsis, pneumonia and IE can be overlooked without diagnostics, which can lead to devastating consequences for the patient (59, 60).

1.3.1 Infective endocarditis

This is a severe infectious disease that can be fatal rapidly if not found and treated properly (60, 61). According to the Global Burden of Disease study of 2017, IE was associated with 2.23 million disability-adjusted life-years (DALYs; 1 DALY represents the loss of the equivalent of 1 year of full health), as a result of death, nonfatal illness or impairment (62). The global changes in DALYs were measured between 2007 and 2017, and decreased among many infectious diseases (for instance lower respiratory tract infections (RTIs), tuberculosis, sexually transmitted infections, enteric infections and meningitis); however, a 17.1% increase in DALYs was seen for IE (62). IE has been shown to be one of the four most common life-threatening infections, together with sepsis, pneumonia and intra-abdominal abscess (63).

IE can cause heart valve destruction, perivalvular abscesses, aneurysms and fistulas, and it is therefore of great importance to diagnose it and initiate antibiotic treatment quickly to avoid the progression of such manifestations (64, 65). Microbiological analysis is important in the diagnostics because most patients with IE present with bacteraemia. The bacterial species causing bacteraemia is considered the most probable cause of IE. However, a positive blood culture is not sufficient for a definitive diagnosis of IE (64, 66). This should be based on modified Duke criteria (Table 1) and defined as definite or possible IE depending on clinical

or pathological fulfilment of the major or the minor criteria (64, 67). For definite IE, two major criteria, or one major criterion and three minor criteria, or five minor criteria should be present. For possible IE, one major and one minor, or three minor criteria should be present (64, 67, 68).

Table 1. The modified Duke criteria for the diagnosis of infective endocarditis (IE)

Major criteria	**Blood cultures positive for IE** Typical microorganisms consistent with IE from two separate blood cultures or Microorganisms consistent with IE from persistently positive blood cultures or Single positive blood culture for *Coxiella burnetii* or phase I IgG antibody titre > 1:800
	Evidence of endocardial involvement ECHO positive for IE (vegetation, abscesses, new partial dehiscence of a prosthetic valve) New valvular regurgitation
Minor criteria	**Predisposition** (heart condition or injection drug use)
	Fever (temperature >38 ºC)
	Vascular phenomena (detected by imaging such as major arterial emboli, septic pulmonary infarcts, infectious aneurysms, intracranial haemorrhage, conjunctival haemorrhages, Janeway's lesions)
	Immunological phenomena (glomerulonephritis, Osler's nodes, Roth's spots, rheumatoid factor)
	Microbiological evidence (positive blood culture not meeting a major criterion or serological evidence of active infection with organisms consistent with IE)

Adapted from (64, 67).

IE requires long antibiotic treatments: 2-6 weeks for native valve IE depending on the causative bacteria and manifestations, and at least 6 weeks for prosthetic valve IE (68, 69). International guidelines for the management of IE suggest that empirical antibiotic treatment is appropriate when IE is suspected, that bactericidal treatment should be used rather than bacteriostatic, and that surgical treatment for IE should be performed when necessary (64).

Clinical and microbiological data of IE from resource-limited settings are scarce, and the disease is commonly underdiagnosed (70-72). Few studies have been conducted, these have shown that the mortality among patients with IE is higher in LMICs compared with high-income countries, with mortality rates of almost 30% (73, 74). Factors contributing to the high mortality rates can be limited diagnostic methods and access to surgical treatment for IE in

resource-limited settings; moreover patients might seek health care late after the onset of IE (61, 73). A major risk factor for IE in LMICs is rheumatic heart disease (RHD), a complication of rheumatic fever affecting the heart valves (74). In high-income countries, RHD has almost disappeared but it is still a problem in many LMICs, contributing to high rates of disability and over a million premature deaths annually (75, 76). India contributes to about 25–50% of the global burden of RHD, indicating that there are many people at increased risk of developing IE in that country (70, 71, 74). However, a study from India also noted that underdiagnosing of IE is a considerable problem (77), indicating that the diagnosis and management of IE in India as well as in other LMICs need to be improved.

1.4 DIAGNOSTICS

To be able to successfully manage infectious diseases and rationalize antibiotic use, there is a need to focus on the development and implementation of diagnostic methods for identifying infectious foci, and verifying pathogens and their resistance patterns (16). In resource-limited settings, access to diagnostic methods can be limited or absent and antibiotics are often prescribed empirically, based on a clinical suspicion of infection (4, 16, 27, 28). Further, limited access to diagnostic methods increases the risk of underdiagnosing infections, which might lead to ineffective treatment.

1.4.1 Microbiological analyses

Diagnostic information about the pathogen that caused the infection is a prerequisite to prescribing a targeted antibiotic treatment for IE. The most common method to identify the pathogen is to send samples for culture, which requires access to a laboratory for microbiological analyses (20). However, results from such analyses usually take a few days, which might cause delays in initiating treatment; this can be associated with increased mortality. Therefore, broad-spectrum antibiotics are often prescribed empirically, while awaiting the response from microbiological analysis, even though it is known that overprescribing of broad-spectrum antibiotics drives antibiotic resistance (7). It is a major challenge to prescribe antibiotics rationally. A study from the USA has shown that clinicians consider that the proper empirical prescribing of antibiotics is difficult if guidelines for treatment pathways do not give explicit recommendations (78). These perceived difficulties arise because recommendations are often written for infections with known bacteria, or because the guidelines are not adapted to local resistance patterns (18, 78). To be able to follow such guidelines, access to microbiological analyses and data on individual pathogen susceptibility to different antibiotics from locally performed blood cultures is necessary and susceptibility tests are required (18). Another obstacle is that prescribers must take the results from the microbiological analysis into account, which is not always done. It is crucial to educate prescribers about the importance of these results, because it can guide treatment and help reduce the unnecessary use of antibiotics (20, 79).

1.4.2 Imaging

In combination with microbiological analysis, diagnostic imaging methods are often recommended to verify the site and manifestations of some infections and can also be used for follow-ups. Depending on the suspected site of infection and the clinical evaluation of the patient, a variety of imaging techniques can be used. In resource-limited settings, access to imaging diagnostics might be limited or absent, because such methods can be costly and labour intensive (80). However, ultrasonography can provide a rapid diagnosis; it does not expose the patient or personnel to ionizing radiation, it can be portable and it requires less training compared with computed tomography and magnetic resonance imaging (80). Therefore, the WHO has pointed out the advantages of ultrasonography in LMICs, which have been confirmed by studies describing its successful use in resource-limited areas (81-84). Ultrasonography can be used to assess infectious processes such as abscesses and fistulas, and echocardiography (ECHO) is suitable for the assessment of cardiac diseases such as IE (64, 65, 85).

In 2019, Murphy et al. published a comparative review of the most recently available guidelines for the management of IE by the Task Force for the Management of IE of the European Society of Cardiology and the American Heart Association IE Writing Committee, (64, 66, 86). The review focusses on the role of imaging in the evaluation and management of patients with IE, and presented conclusive recommendations for the initial assessment of patients with clinically suspected IE using ECHO (86). ECHO can be used to identify one or more of the typical manifestations of IE such as vegetation, abscesses, aneurysms, destruction and fistulas (64). Figure 1 shows normal mitral and aortic valve morphology compared with mitral valve vegetation and aortic valve vegetation and destruction. ECHO can be conducted using a transoesophageal (TEE) or a transthoracic (TTE) approach, but the TEE method often provides better image quality, which increases the chance of finding infections. Microbiological analysis is an important step to advance the diagnosis of a patient with suspected IE. However, a positive culture confirming bacteraemia in itself is not enough to confirm this (64, 69). To confirm the diagnosis, one or more infectious manifestations need to be verified by ECHO, and many hospitals use this in daily clinical work, together with microbiological analysis to identify and diagnose IE (64, 65, 68). It should be noted that according to international guidelines for the treatment of IE, antibiotic treatment is recommended if manifestations of IE are found on ECHO, and also if the results from microbiological analyses of blood samples are negative (64, 65, 87). Identification of aetiology and cardiac IE manifestations are both crucial for the diagnosis and treatment of IE, yet the associations between microbiological data and ECHO findings need to be further explored (88-90).

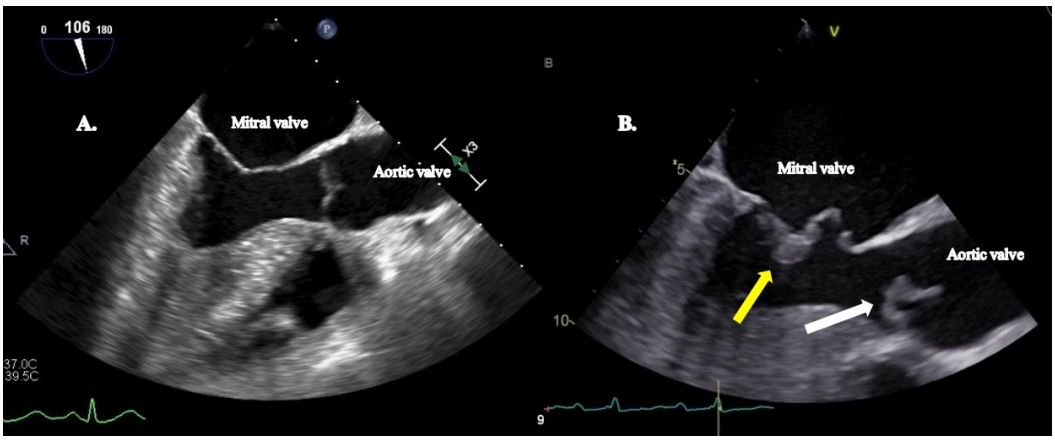

Figure 1. Normal mitral and aortic valve morphology compared with mitral valve vegetation and aortic valve vegetation and destruction. A. shows normal mitral and aortic valves. B. shows vegetation on the mitral valve (yellow arrow) and vegetation and valvular destruction on the aortic valve (white arrow) caused by coagulase-negative staphylococci detected by transoesophageal echocardiography.

1.5 THESIS RATIONALE

To ensure long-term, successful prevention and treatment of infectious diseases and to retain antibiotics as available, effective and safe treatments with quality-assured medications, the WHO Global Action Plan on antimicrobial resistance requests all Member States to "monitor and promote optimization of antimicrobial use at national and local levels" (10). The aims of monitoring and promotion are to assess how antibiotics are being used by understanding when, how and why antibiotics are used irrationally, to improve the practices of antibiotic prescribers, dispensers and consumers, to assess the impact of interventions aiming to improve antibiotic use and to understand the relationship between antibiotic use and the spread of antibiotic resistance.

In resource-limited settings, the burdens of infectious diseases and antibiotic resistance are both high. Today, the irrational use of antibiotics as well as the underdiagnosis of infections are common in resource-limited settings in India, contributing to unnecessarily high mortality rates from infectious diseases and to antibiotic resistance (38, 40, 41). To combat the burden of infectious diseases, the rational use of antibiotics and management of infectious diseases is required. To rationalize antibiotic use, it is necessary to map antibiotic prescribing practices and to identify areas for improvements. Historically, this has been difficult in resource-limited settings, as the economic conditions for health care are often low and patient records might not be computerized; hence data collection and mapping of antibiotic use can be challenging (22). By monitoring antibiotic use in a resource-limited setting in India, needs for improvements can be identified and provide the basis for future interventions.

The challenges to appropriate use of antibiotics in resource-limited settings is aggravated by the lack or absence of diagnostic tools to assist clinicians in their clinical decision-making (91). There is a need to highlight the importance of diagnostic methods that can be used for identification and verification of infectious diseases in resource-limited settings. Diagnostic

methods can contribute with aspects such as individual evaluation of the disease and identification of complications which might be valuable for the clinicians in the management and treatment of patients with infectious diseases. Evaluating diagnostic methods that can be implemented and used in resource-limited settings is a way to get closer to optimized management and treatment of patients with infectious diseases in such settings. Initial evaluation of a diagnostic method in a high-income setting, where the method is fully implemented and of good quality, enables safe evaluation before implementation in resource-limited settings.

In areas with lack of access to diagnostics, there is a risk of underdiagnosing infectious diseases. IE is often underdiagnosed in such settings. Evaluating the significance of ECHO for the diagnosis of IE in a high-income setting, can be valuable for the implementation of ECHO and the management of patients with IE in resource-limited settings.

2 AIMS

The overall aims of **Papers I-III** were to map and describe antibiotic prescribing practices for in-patients with infectious diseases in a resource-limited setting in India. A further aim of **Papers I-III** was to identify areas of improvements in the antibiotic prescribing practices and management of infectious diseases in such settings. Limited access to diagnostic methods complicates the management of infectious diseases in resource-limited settings and increases the risk of disregarding severe infectious diseases, such as IE. Therefore, it is important to evaluate diagnostic methods that can improve the management of infectious diseases in such settings. **Paper IV** focuses on analysing ECHO as a diagnostic method for IE. The aim was to assess whether specific manifestations detected by ECHO were associated with certain bacterial species in patients with IE, to evaluate whether the ECHO findings could give a suggestion of the bacterial aetiology in these patients. The findings could add aspects to the management and treatment of patients with IE. **Paper IV** was conducted in a high-income setting in Sweden, where a national registry of patients with IE was available, and where both microbiological analyses and ECHO were performed. The findings could possibly be useful also in resource-limited settings.

The specific aims were as follows:

- To describe and compare antibiotic prescribing practices in the medical ICUs (**Paper I**) and the paediatric departments (**Paper II**) respectively, at two hospitals in a resource-limited setting in India.
- To present the antibiotic prescribing practices among in-patients with severe infections over a 10-year period at two hospitals in a resource-limited setting in India (**Paper III**).
- To evaluate associations between specific IE manifestations detected by ECHO, and bacterial species in patients with IE in a high-income setting in Sweden (**Paper IV**).

3 METHODS

3.1 STUDY SETTINGS

3.1.1 Ujjain

Papers I–III are based on studies conducted in the Ujjain district of Madhya Pradesh, India (Figure 2). This district has approximately 2 million inhabitants, of whom approximately 61% live in rural areas and villages (92). Madhya Pradesh is less economically and socially developed, with lower health-care performance and higher infant and maternal mortality, than many other states of India. In 2017, India had an overall infant mortality rate (death before the age of 1 year) of 36 per 1000 reported live births, and Madhya Pradesh had an infant mortality rate of 41.9. The infant mortality rate in the Ujjain district was 28.7 (93, 94). The predominant risk factors for infant mortality in Madhya Pradesh and Ujjain are child and maternal malnutrition (93). In India, the number of years of healthy life expectancy in 2016, which corresponds to the number of years a person is expected to continue to live in a healthy condition at birth, was 59.9 years for women and 58.7 years for men (95). In 2011, the calculated number of hospital beds per 10,000 people in India was 7 (for reference, globally the number of hospital beds per 10,000 people in 2011 was 27) (96).

Figure 2. Map of India (yellow), Madhya Pradesh (red) and the Ujjain district (black).

The studies were conducted in two tertiary care, private sector hospitals run by the same charitable trust, a non-teaching hospital (NTH) and a teaching hospital (TH). When **Paper I and II** were conducted, the NTH had 350 beds and the TH had 570 beds. When **Paper III** was conducted, the NTH had 400 beds and the TH had 800 beds. The NTH is located centrally in

Ujjain city while the TH is located in a rural area of the Ujjain district. At the NTH, medical services are charged but at a reduced level and the patients must also purchase their medicines during hospital stay. At the TH, patients are provided with medical services and medicines free of charge. Representatives from pharmaceutical companies can visit the prescribers at the NTH but not at the TH. At the time when the data was collected (2008–2017), no local prescribing guidelines were available at the hospitals; however, a local essential medicines list was available at the TH, although it was not implemented completely. Samples were not sent routinely for microbiological analyses (culture) at either of the hospitals and diagnostic imaging methods were limited.

3.1.2 Stockholm

Paper IV is based on data from patients admitted to the Karolinska University Hospital (KUH) in Stockholm, Sweden. On 31 March 2020, Stockholm county had a population of approximately 2.4 million (97). The estimated infant mortality rate in Stockholm county was 1.9 deaths per 1000 live births in 2017 (98). By comparison, the overall infant mortality rate in Sweden was 2.1 deaths per 1000 live births in 2019 (99). In 2016, Sweden had the greatest number of healthy years of life expectancy among the European countries (73.3 years for women and 73.0 years for men) (100). There were 26 hospital beds per 10,000 people in Sweden in 2013 (96).

The study was conducted at the KUH in Stockholm, which is one of the largest university hospitals in Europe (101). Here, both ECHO and microbiological analysis are used routinely among patients with IE and both techniques are of high quality. There is a Department of Clinical Physiology specialized in cardiac imaging with a large focus on the latest technology in ECHO. In compliance with guidelines for the management of IE, there is a multidisciplinary team of physicians from the departments of Infectious Diseases, Clinical Physiology, Cardiology, and Thoracic Surgery that manage the patients with definite or possible IE (64).

3.2 OVERVIEW OF PAPERS

An overview of the papers arising from this thesis is presented in Table 2.

Table 2. Overview of papers and methods

Paper	I	II	III	IV
Topic	AB prescribing in intensive care units	AB prescribing in paediatric departments	Empirical AB prescribing to patients with severe infections in 2008–2017	Associations between ECHO manifestations and bacteria in patients with IE
Design	Prospective cross-sectional study	Prospective cross-sectional study	Time series analysis	Registry-based cohort study
Data sources	Data collection from the NTH and TH in Ujjain	Data collection from the NTH and TH in Ujjain	Data collection from the NTH and TH in Ujjain	Swedish National Registry of Infective Endocarditis
Study population	In-patients at medical ICUs (all ages) 4,843 patients	In-patients at paediatric departments (<18 years) 6,993 patients	In-patients (≥18 years) with severe infectious diseases 3,766 patients	In-patients (≥18 years) with definite IE at KUH, Stockholm 492 patients
Study period	1 April 2008 to 31 March 2011	1 April 2008 to 31 March 2011	1 April 2008 to 31 May 2017	1 Jan 2008 to 31 Dec 2017
Exposure	Any infectious diagnosis or presumed symptoms of infection or no infectious diagnosis	Acute gastroenteritis, respiratory tract infections, enteric fever, viral or unspecified fever	Epiglottitis, pneumonia, peritonitis, pyelonephritis, cellulitis, erysipelas, septic arthritis, IE, meningitis, sepsis	Bacteria found in blood culture or culture/PCR from surgical material
Covariates	Age, sex, at the TH or NTH	Age, sex, at the TH or NTH	Year of AB prescription at the TH or NTH	Native versus prosthetic valves, IV drug abuse, bicuspid aortic valve
Outcomes	AB prescription	AB prescription	AB prescription	IE manifestations detected by ECHO
Main statistical analyses	Chi-squared test, Student's t test	Chi-squared test, Student's t test	Linear regression, chi-squared test	Logistic and linear regression, Student's t test, chi-squared test

AB, antibiotic; ECHO, echocardiography; ICU, intensive care unit; IE, infective endocarditis; IV, intravenous; KUH, Karolinska University Hospital; NTH, non-teaching hospital; PCR, polymerase chain reaction; TH, teaching hospital.

3.3 DATA SOURCES

3.3.1 Data of patients at the NTH and the TH in Ujjain

For **Papers I-III**, patient data were collected manually from the NTH and the TH in Ujjain by specially trained nursing staff. Neither of the hospitals had computerized prescribing records. A form was developed to record antibiotic prescribing for each patient at the hospitals. This was attached to each patient's file at admission to the hospital and was updated daily until discharge from hospital or death. The form included the patient's age, sex, department number, registered diagnoses and dates for admission and discharge. If antibiotics were prescribed, the form included what antibiotic had been prescribed with its generic and trade name, route of administration, dose, frequency and date of prescription. To minimize the risk of missing data, the form was printed on pink paper to be visible among others in the patient's file. As of 2008, data from the forms were entered to a file by trained computer operators.

For **Paper I**, all patients admitted to the medical ICUs at either the NTH or TH who stayed at least one night in hospital between 1 April 2008 and 31 March 2011 were included. At the NTH there was a separate ICU for neonatal patients, who were not included in the study. At the TH, there were separate ICUs for medical patients, surgical patients, paediatric patients and neonatal patients. In this study, only data for the patients from the medical ICU were included. Some paediatric patients were admitted to the medical ICU, so were included. Of the 6,141 patients admitted to the medical ICUs (4,338 from the NTH and 1,803 from the TH), 4,843 were included, 3,472 from the NTH and 1,371 from the TH.

For **Paper II**, all patients younger than 18 years of age who stayed for at least one night at either of the paediatric departments at the NTH or the TH between 1 April 2008 and 31 March 2011 were analysed. Of the 6,825 patients admitted to the paediatric departments during this period, 4,848 from the NTH and 1,977 from the TH were included.

For **Paper III**, data were included for patients aged 18 years and older, admitted to the NTH and the TH between 1 April 2008 and 31 March 2017 and who stayed at least one night, with one of the following diagnoses (which the WHO lists as indications for empirical treatment): epiglottitis, pneumonia, peritonitis, pyelonephritis, cellulitis, erysipelas, septic arthritis, IE, meningitis and sepsis (25). Of the 243,774 patients admitted to the hospitals during this period (134,666 from the NTH and 109,108 from the TH), 3,766 were included: 2,504 from the NTH and 1,262 from the TH.

3.3.2 Swedish National Registry of Infective Endocarditis

For **Paper IV**, data was obtained from the Swedish National Registry of Infective Endocarditis (SRIE), which was established in 1995. Since 2008, data from patients treated for definite or possible IE (International Classification of Diseases codes I33.0, I33.9, I38.9 and I39.8) has been reported online by all Swedish departments for infectious diseases. The SRIE's coverage for patients with IE is estimated to be 85% (when compared with the Swedish health-care statistics network of registered diagnoses in 2016) (102). Data in the registry include age, sex,

results from blood cultures, and/or polymerase chain reaction (PCR) amplification results from material obtained during cardiac surgery, ECHO findings, whether antibiotics had been prescribed, duration of antibiotic treatment, in-hospital mortality (defined as death during hospital stay) as well as risk factors such as intravenous (IV) drug abuse, and the presence of prosthetic cardiac or bicuspid aortic valves.

Data from 593 patients at the age of 18 years or older, admitted to the Department of Infectious Diseases at the KUH with definite IE between 1 January 2008 and 31 December 2017 were obtained from the SRIE. Patients with both negative blood cultures and negative cultures from material obtained during cardiac surgery for IE were excluded, as were patients who did not undergo ECHO during hospital stay. In total, 492 patients with a definite diagnosis of IE were included in the study.

3.4 METHODOLOGY FOR CLASSIFICATION OF ANTIBIOTICS

For **Papers I-III**, the antibiotics were classified by generic names and the Anatomical Therapeutic Chemical (ATC) classification system and antibiotic prescribing was measured by calculating defined daily doses (DDDs) according to the WHO Collaborating Centre for Drug Statistics Methodology (WHOCC) (103). The purpose of using the ATC/DDD methodology was to monitor the prescribing or use of medicines effectively, and to combine and compare different substances. By using this internationally recognized method, medicine prescribing can be compared between different centres, both at local and international levels, and it is possible to study time trends in the use of a specific medicine. The ATC system classifies antibiotics into several subcategories, and for each substance there is a given DDD that corresponds to the assumed daily maintenance dose if a medicine is used for its main indication in adults (Table 3). DDD is used as a measurement to compare the exposure or therapeutic intensity of a medicine in a defined population between different time periods or between different population groups (103).

The ATC/DDD methodology includes most of the medicines available on the market and is often updated for new medicines being developed and marketed. During the course of the thesis, the list of codes was updated, and it now contains several FDCs of antibiotics, that were not registered in the system at the time of the first two studies. For FDCs of antibiotics where no ATC codes were available, the codes were generated based on advice from the WHOCC (28). According to the WHOCC, oral metronidazole is coded as an anti-protozoal (P01) agent (103), but according to the National List of Essential Medicines in India (NLEMI), oral metronidazole is considered as an antibacterial for anaerobic infections (104). Thus, for **Papers I and II**, in which the antibiotic prescribing was related to the adherence for NLEMI, oral metronidazole was analysed as an anti-infective agent (J01) although it was coded as P01AB01 (104).

To preserve the effectiveness of antibiotic treatment and improve clinical outcomes, the WHO has adopted a classification of antibiotics that suggests how their use should be prioritized: the so-called *access*-, *watch*- and *reserve* antibiotic categories (10, 25, 36). *Access* antibiotics (first-

line antibiotics) should be widely available, affordable and of good quality; *watch* antibiotics (second-line antibiotics) include most of the highest priority, critically important medicines and should be used only for specific and limited indications; and *reserve* antibiotics (third-line antibiotics) should only be used when all alternative antibiotics have been shown to be unsuccessful (10, 25, 36).

Table 3. Example of classification of an antibiotic according to the ATC/DDD classification methodology

Group	J	Anti-infectives for systemic use
Subgroups	J01	Antibacterials for systemic use
	J01M	Quinolone antibacterials
	J01MA	Fluoroquinolones
ATC code	J01MA02	Ciprofloxacin, DDD oral: 1 g DDD parenteral: 0.8 g

Adapted from (103). For antibiotic classification, the 2019 ATC/DDD classification was used. ATC, Anatomical Therapeutic Chemical; DDD, defined daily dose.

3.5 DATA ANALYSIS

3.5.1 Paper I – Antibiotic prescribing in intensive care units

All included patients were divided into three groups at each hospital according to their registered diagnosis: group 1, patients with registered infectious diagnoses; group 2, patients with diagnoses with presumed symptoms of infections, i.e. not registered with any infectious diagnosis but with symptoms of infections, for example fever; and group 3, patients registered with diagnoses not associated with infection. Patient data were analysed for age (divided into four age groups), sex, duration of hospital stay and whether antibiotics were prescribed during their hospital stay. The antibiotic prescription data were analysed for type of antibiotic, dose, treatment duration, frequency and route of administration and and–if it was prescribed–by generic or trade name. The total numbers of DDDs for prescribed antibiotics were calculated and compared. In addition, adherence to the WHO List of Essential Medicines (WHOLEM) and to NLEMI were evaluated.

3.5.2 Paper II – Antibiotic prescribing in paediatric departments

Among all paediatric patients, the five most common diagnoses related to infectious diseases– or to symptoms commonly associated with infectious diseases–were calculated. The patients were divided into five groups according to the most commonly registered infection-related diagnoses: acute gastroenteritis including bloody diarrhoea; RTIs including both upper and lower RTIs, pneumonia and bronchitis; enteric fever; viral or unspecified fever. Patient data were analysed for sex, duration of hospital stay and whether antibiotics were prescribed during hospital stay. The antibiotic prescription data were analysed for type of antibiotic, dose, treatment duration, frequency and route of administration and whether the antibiotic was prescribed by generic or trade name as well as the total number of DDDs per prescribed antibiotic. The drug utilization 90% methodology was used to present the prescribing of antibiotics accounting for 90% of all antibiotics prescribed (105). Further, adherence to the Indian Academy of Paediatrics List of Essential Medicines (IAP-LEM) was evaluated (106).

3.5.3 Paper III – Empiric antibiotic prescribing for severe infections in 2008–2017

The included patients were analysed at group level depending on their registered diagnosis: epiglottitis, pneumonia, peritonitis, pyelonephritis, cellulitis, erysipelas, septic arthritis, IE, meningitis and sepsis; however, few patients were registered with epiglottitis, pyelonephritis, erysipelas, septic arthritis, IE or meningitis, so data from patients with pneumonia, peritonitis, cellulitis and sepsis were selected for detailed analysis. Patient data were analysed for age, sex, duration of hospital stay and whether antibiotics were prescribed. The antibiotic prescription data were analysed for type of antibiotic, dose, treatment duration, frequency, number of prescriptions per patient as well as DDDs. The antibiotic prescribing was measured in DDDs per 1,000 patient days using the following formulae:

DDD per prescription = dose in grams * frequency/WHO DDD for the prescribed antibiotic

DDD per 1000 patient days = (DDD_{total} * 1000/365)/N),

where DDD_{total} is the total antibiotic prescribing (in DDDs) prescribed during 1 year among a patient group and N is the total number of patients in that group during that year.

For antibiotic classification, the 2019 ATC/DDD classification was used (103). Antibiotics were grouped according to the first 4–5 characters of their ATC code: J01A, J01B, J01C group 1 (including all antibiotics in J01CA–J01CG), J01CR, J01D, J01E, J01F, J01G, J01M, J01R and J01X (Table 3). The antibiotics were also classified for: *access*, *watch*, *reserve* and FDCs of antibiotics according to the WHO (10, 25, 36). Some of the prescribed antibiotics were not categorized specifically by the WHO but if their antibiotic groups were classed, these antibiotics were added to the relevant category according to their antibiotic group. For example, cefuroxime was not categorized by the WHO, but second generation cephalosporins were categorized as *access* antibiotics, and because cefuroxime is a second generation cephalosporin, it was added to the *access* category for analysis (107). The following antibiotics

were added in the *access* category: first generation cephalosporins (cefadroxile, cefradine); second generation cephalosporins (cefuroxime, cefaclor); aminoglycosides (netilmicin, streptomycin, kanamycin, tobramycin); and ornidazole, tinidazole, piperacillin and tazobactam. For the *watch* category: third generation cephalosporins (cefoperazone, cefodoxime); quinolones and fluoroquinolones (ofloxacin, gemifloxacin, gatifloxacin, pazufloxacin, prulifloxacin); and finally the macrolid roxithromycin. Because the FDCs of antibiotics consist of at least two antibiotics, often from different categories, FDCs were added as a separate category (Appendix I).

3.5.4 Paper IV – Associations between ECHO manifestations and bacteria in patients with IE

The patients were divided into groups according to the bacteria registered as possible infectious pathogens causing IE in each patient. The primary pathogen was considered to be the bacterial strain identified from positive blood culture results, and the secondary pathogen was considered to be the bacterial strain cultured from material sampled during cardiac surgery or identified by PCR amplification. In total, 490 patients had positive blood culture results, and two patients had negative blood culture results but positive cultures or PCR amplification from material sampled during cardiac surgery; in total, 492 patients were included. Among patients with positive blood culture results, 19 were registered as having more than one bacterial species (or bacteria plus fungi); in such cases the first pathogen listed was chosen for analysis. Patient data were analysed for age, sex, bacterial pathogen causing the IE as explained above, any history of IV drug abuse, bicuspid aortic valve, prosthetic or native cardiac valve, surgical treatment for IE, in-hospital mortality as well as for the manifestations and total numbers of manifestations of IE detected by ECHO. The IE manifestations detected by ECHO were divided into groups depending on their location and morphology: aortic valve vegetation; mitral valve vegetation; tricuspid valve vegetation; pulmonary valve vegetation; cardiovascular implantable electronic device (CIED)-associated IE; ventricular septal defect; and perivalvular abscess. The manifestations were analysed both separately and together, among those patients with more than one registered ECHO manifestation.

3.5.5 Statistical analyses

Data were analysed using Stata version 13.0, 15.0 and 16.0 (Stata Corp., College Station, TX, USA), IBM SPSS statistics version 22.0 (IBM Corp., Armonk, NY, USA) and Excel (Microsoft Corp., Redmond, WA, USA). Descriptive analyses were conducted for all studies and are presented with frequencies and percentages for categorical variables and means and standard deviations (SDs) or medians and 25^{th} and 75^{th} percentiles for continuous variables. All statistical tests were two-tailed.

For **Papers I and II**, chi-squared tests (for numbers ≥ 5) and Fischer's exact test (for numbers < 5) were used for comparing categorical variables. For **Papers I and II,** when comparing continuous variables, independent samples t tests were used because the variables were distributed normally at both hospitals. For **Paper I**, Bonferroni's correction for multiple

comparisons was applied and an adjusted significance level of 0.001 was calculated, therefore, P values ≤ 0.001 were considered significant. For **Paper II**, P values < 0.05 were considered significant.

For **Paper III**, linear regression was used for comparing continuous variables. For comparing categorical values, chi-squared tests (for values ≥ 5) and Fischer's exact test (for values < 5) were applied and the results were presented as odds ratios (ORs) and 95% confidence intervals (CIs). Time series analysis of antibiotic prescribing from 2008 to 2017 was performed by linear regression, with DDDs per 1,000 patient days as the dependent variable and the year as the independent variable, to obtain a slope for the trend. Time series analyses were conducted for the prescribing of antibiotics in the "*access, watch, reserve*, and FDCs of antibiotics" categories as well as for antibiotic prescribing among the five most common diagnoses: sepsis, cellulitis, pneumonia, peritonitis and meningitis. P values < 0.05 were considered significant.

For **Paper IV**, logistic regression, chi-squared tests (for values ≥ 5) and Fisher's exact test (for values < 5) were conducted for comparing categorical values and are presented with ORs and 95% CIs. Linear regression and Student's t tests were used to compare continuous variables and skewed variables were log-transformed to normalize them before analyses. Linear and logistic regression analyses were used for multivariable analysis and linear regression was used for secular trend analysis. P values < 0.05 were considered significant.

3.6 ETHICAL CONSIDERATIONS

Papers I-III include data from patients admitted to the two hospitals in Ujjain, India. Data from patients included in **Papers I-III** were collected by trained nurses at the two hospitals. The patients were informed about the research project at the time of admission to hospital. The form for data collection included information about whether the patient was prescribed antibiotics and, if so, information about the particular treatment. The forms from the hospitals were collected regularly by a person involved in the project and the information was entered to a computerized file. The forms were kept secure by the research group in Ujjain. Data were registered anonymously and since there were no personal identity numbers entered in the forms. For anonymizing, a unique code was given for each patient form with no possibility to track the data to an individual person's identity. Hence, the studies are unlikely to harm the patients' integrity. Because these studies were observational, they did not affect or cause any changes in the patients' treatments and no additional examinations of the patients were conducted.

Paper IV covers data from patients who attended the Department of Infectious Diseases at the KUH, obtained from the SRIE. The patients were informed about the registry during their hospital stay, and that the data might be used in further research. The study did not imply any risks for the patients because it did not affect or cause any changes in the patients' treatments and no additional examinations of the patients were conducted. Ethical aspects that the study could implicate are primarily related to personal integrity and data security. Using data from the existing registry implies minimal risk for the included patients, and intrusion to personal

integrity was eliminated by anonymizing the data. Data from the SRIE were obtained in anonymized form; this is possible without prior consent according to the Swedish Data Protection Authority (108). The risk of data dissemination was minimal because both ethical approval from the Regional Ethics Review Board and permission from the Registry Board of the SRIE were required to obtain and use the data from the SRIE.

The studies did not directly benefit the included patients, but the study results might lead to improvements in the management of patients in the hospitals, both in Ujjain and in Stockholm. Presentation of the results from the studies in Ujjain could lead to improved practices and routines in antibiotic use and prescribing, and thus contribute to reducing the risk of development of antibiotic resistance. Presentation of the results from the study in Stockholm might lead to improvements in the management of patients with IE, possibly with faster diagnostics and shorter times to adequate antibiotic treatment.

Papers I–III were approved by the Ethics Committee of Ruxmaniben Deepchand Gardi Medical College, Ujjain with approval numbers: 41/2007, 114/2010, and 311/2013, respectively. **Paper IV** was approved by the Regional Ethics Review Board, Stockholm, Sweden (number K 2018-6018).

4 MAIN RESULTS

The main results of **Papers I-IV** have been integrated in this section, under appropriate headings.

4.1 ANTIBIOTIC PRESCRIBING PRACTICES

Table 4 shows the numbers of included patients, patients prescribed antibiotics and duration of hospital stay, and antibiotic treatment of patients from the medical ICUs (**Paper I**) from the paediatric departments (**Paper II**) and among patients registered with severe infections (**Paper III**) in the NTH and TH.

Table 4. Antibiotic prescribing details at medical ICUs and paediatric departments at the NTH and TH in Ujjain, India

	Paper I, medical ICUs			**Paper II, paediatric departments**			**Paper III, patients with severe infections**		
	NTH	**TH**	**P value**	**NTH**	**TH**	**P value**	**NTH**	**TH**	**P value**
Included patients, n (%)	3472 (100)	1371 (100)		4848 (100)	1977 (100)		2504 (100)	1262 (100)	
Female patients, n (%)	1290 (37)	580 (42)		1418 (29)	842 (43)		894 (36)	301 (24)	
Male patients, n (%)	2182 (63)	791 (58)		3430 (71)	1135 (57)		1610 (64)	961 (76)	
Patients prescribed antibiotics, n (%)	2474 (71)	965 (70)	0.548[a]	3963 (82)	679 (34)	**<0.001[a]**	2294 (92)	1122 (89)	**<0.05[a]**
Female patients prescribed antibiotics, n (%)	988 (77)	394 (68)	**<0.001[a]**	1127 (79)	**296 (35)**	**<0.001[a]**	809 (90)	259 (86)	**0.03[a]**
Male patients, prescribed antibiotics, n (%)	1486 (68)	571 (72)	0.033[a]	2836 (83)	**383 (34)**	**<0.001[a]**	1485 (88)	863 (90)	**0.03[a]**
Duration of hospital stay, mean days (±SD)	2.6 (3.1)	4.9 (5.9)	**<0.001[b]**	4.5 (3)	6.7 (5.7)	**<0.001[b]**	4.4 (4.8)	10.1 (10.5)	**<0.001[b]**
Duration of antibiotic treatment, mean days (±SD)	3.2 (2.5)	4.9 (4.3)	**<0.001[b]**	4.4 (2.7)	6.6 (4.6)	**<0.001[b]**	**1.1 (0.35)**	**9.4 (8.9)**	**<0.001[b]**

[a]P values were obtained using Pearson's chi-squared test, comparing the distributions of patients prescribed antibiotics between the two hospitals. [b]P values were obtained from linear regression, comparing the mean days of hospital stay or antibiotic treatment duration between the two hospitals. Significant P values are shown in bold type. ICU, intensive care unit; NTH, non-teaching hospital; SD, standard deviation; TH, teaching hospital.

Most of the patients registered with infection-related diagnoses at the medical ICUs were prescribed antibiotics at both hospitals (among patients with infections, 93% of the 400 at the NTH were prescribed antibiotics and 89% of the 189 at the TH, P = 0.030) (**Paper I**). Further, most patients in the medical ICUs with no infection-related diagnoses were prescribed antibiotics at both hospitals. Antibiotics were also prescribed to a high extent at both hospitals for presumed symptomatic reasons (Table 5). The most commonly prescribed antibiotics (comprising ≥ 95% of all antibiotic prescriptions at either hospital) are presented in Table 6.

Table 5. Antibiotic prescribing details at medical ICUs in the NTH and the TH in Ujjain, India (Paper I)

Patient details	**NTH**	**TH**	**P value[a]**
Patients with infections, n	400	189	0.030
Patients with infections prescribed antibiotics, n (%)	373 (93)	168 (89)	0.071
Patients with presumed symptoms of infections, n	319	151	**<0.001**
Patients with presumed symptoms of infections prescribed antibiotics, n (%)	297 (93)	129 (85)	0.008
Patients with diagnoses not associated with infections, n	2753	1031	**<0.001**
Patients with diagnoses not associated with infections prescribed antibiotics, n (%)	1822 (66)	668 (65)	0.671
Antibiotic prescription details	**NTH**	**TH**	**P value[a]**
Total number of antibiotic prescriptions, n	10371	6647	
Antibiotic prescriptions by generic name, n (%)	272 (3)	733 (11)	**<0.001**
Antibiotic prescriptions by parenteral route of administration, n (%)	10081 (97)	5357 (81)	**<0.001**
Antibiotic prescriptions adherent to WHOLEM, n (%)	4847 (47)	3437 (52)	**<0.001**
Antibiotic prescriptions adherent to NLEMI, n (%)	5509 (53)	4654 (70)	**<0.001**

[a]P values were obtained using Pearson's chi-squared test, comparing the distributions of patients or prescriptions between the two hospitals. Significant P values are shown in bold type. ICU, intensive care unit; n, number; NLEMI, National List of Essential Medicines in India; NTH, non-teaching hospital; SD, standard deviation; TH, teaching hospital; WHOLEM, World Health Organization List of Essential Medicines.

Table 6. The most commonly prescribed antibiotics at medical ICUs at the NTH and the TH in Ujjain, India (Paper I)

ANTIBACTERIALS FOR SYSTEMIC USE: J01	NTH	TH	P value[a]
Tetracyclines: J01AA, n (%)	1 (0)	196 (3)	**<0.001**
Extended-spectrum penicillins: J01CA, n (%)	152 (1)	270 (4)	**<0.001**
Combination of penicillins including β-lactamase antibiotics: J01CR, n (%)	1963 (19)	1137 (17)	0.003
3rd generation cephalosporins: J01DD, n (%)	2935 (28)	2096 (32)	**<0.001**
Carbapenems: J01DH, n (%)	176 (2)	2 (0)	**<0.001**
Other aminoglycosides: J01GB, n (%)	350 (3)	295 (4)	**<0.001**
Fluoroquinolones: J01MA, n (%)	512 (5)	1625 (24)	**<0.001**
Combination of antibiotics: J01RA, n (%)	3050 (29)	102 (2)	**<0.001**
Imidazole derivatives: J01XD, n (%)	877 (8)	703 (11)	**<0.001**
AGENTS AGAINST AMOEBIASIS & OTHER PROTOZOAL DISEASES: P01			
Nitroimidazole derivatives: P01AB, n (%)	2 (0)	170 (3)	**<0.001**
Total number of prescriptions, n	10371	6647	

[a]P-values were calculated using Pearson's chi-squared test and Fischer's exact test (for values <5), comparing the distribution of prescriptions between the two hospitals. Significant P values are shown in bold type. ICU, intensive care unit; NTH, non-teaching hospital; TH, teaching hospital.

4.2 FOCUS OF INFECTION

Among patients in medical ICUs who were admitted to the NTH and TH from 2008 to 2011, 400 (12%) of the 3,472 patients at the NTH and 189 (14%) of the 1,371 patients from the TH were registered with infectious diagnoses (**I**). The most common infectious diagnoses at the ICUs were gastrointestinal infections, meningitis, RTIs, sepsis, tuberculosis and urinary tract infections. Antibiotic prescribing rates and compliance with the WHOLEM and NLEMI among these patients are presented in Table 7.

Table 7. Antibiotic prescribing among patients with selected infections at medical ICUs in the NTH and the TH in Ujjain, India (Paper I)

Diagnosis	Patients, n (%) of patients prescribed antibiotics		P value[a]	Antibiotic prescriptions, n (%) of prescriptions adherent to WHOLEM or NLEMI		P value[a]
	NTH	**TH**		**NTH**	**TH**	
Gastrointestinal infections	33 (79)	36 (81)	0.855	89 (51)	192 (89)	**<0.001**
Meningitis	30 (100)	3 (100)	-	135 (39)	60 (68)	**<0.001**
Respiratory tract infections	54 (96)	44 (93)	0.403	213 (31)	270 (30)	0.665
Sepsis	93 (94)	10 (90)	0.522	417 (41)	56 (73)	**<0.001**
Tuberculosis	96 (96)	62 (89)	0.083	338 (58)	397 (62)	0.272
Urinary tract infections	20 (100)	20 (90)	0.244	86 (52)	101 (75)	**0.001**

[a]P-values were calculated using Pearson's chi-squared test or Fischer's exact test (for values <5), comparing the distribution of patients prescribed antibiotics or antibiotic prescriptions adherent to the WHOLEM or NLEMI, between the two hospitals. Significant P values are shown in bold type. ICU, intensive care unit; NLEMI, National List of Essential Medicines in India; NTH, non-teaching hospital; TH, teaching hospital; WHOLEM, World Health Organization List of Essential Medicines.

Among the patients admitted to the paediatric departments in 2008–2011, the most common registered diagnoses related to infections were: acute gastroenteritis (including bloody diarrhoea), RTIs (including upper and lower respiratory tract infections, pneumonia and bronchitis), enteric, viral or unspecified fever (**Paper II)**. Antibiotic prescribing rates and details in the diagnosis groups among paediatric patients are presented in Table 8 and the most commonly consumed antibiotics (measured in DDD per 1000 patients) are presented in Table 9. Among the paediatric patients, less than 40% of the antibiotic prescriptions for paediatric patients were adherent to the Indian Academy of Paediatrics List of Essential Medicines (IAP-LEM); adherence to the IAP-LEM was significantly higher at the TH compared with the NTH (prescriptions adherent to IAP-LEM: NTH 24% vs. TH 37%; $P < 0.001$).

Table 8. Antibiotic prescribing among paediatric patients with diagnoses related to infections in the NTH and TH in Ujjain, India (Paper II)

Diagnosis	Hospital	Patients, n	Patients prescribed AB, n (%)	Duration of hospital stay, mean days (±SD)	Duration of AB treatment, mean days (±SD)	AB prescriptions, n (% prescribed by generic name)	Number of different ABs prescribed in each diagnosis group, n
GE	NTH	925	695 (75)	3.55 (1.87)	3.50 (1.79)	3077 (5)	95
	TH	168	51 (30)	5.68 (4.33)	4.78 (2.30)	356 (64)	18
P value			**<0.001[a]**	**<0.001[b]**	**<0.001[b]**	**<0.001[a]**	
RTIs	NTH	1017	940 (92)	4.40 (2.12)	4.32 (1.80)	5023 (2)	111
	TH	166	92 (55)	6.58 (4.67)	6.42 (3.67)	1039 (65)	27
P value			**<0.001[a]**	**<0.001[b]**	**<0.001[b]**	**<0.001[a]**	
Enteric fever	NTH	218	205 (94)	4.79 (2.03)	4.75 (2.09)	1223 (1)	62
	TH	41	33 (80)	7.59 (4.25)	6.70 (2.02)	264 (74)	9
P value			**0.008 [a]**	**<0.001[b]**	**<0.001[b]**	**<0.001[a]**	
Viral fever	NTH	185	130 (70)	3.86 (1.94)	3.76 (1.59)	532 (3)	53
	TH	101	32 (32)	5.87 (3.65)	5.27 (2.23)	212 (69)	17
P value			**<0.001[a]**	**<0.001[b]**	**0.001[b]**	**<0.001[a]**	
Fever	NTH	134	118 (88)	3.48 (1.43)	3.47 (1.48)	469 (1)	45
	TH	34	16 (47)	7.29 (6.31)	4.65 (3.25)	178 (77)	11
P value			**<0.001[a]**	**0.001[b]**	0.127[b]	**<0.001[a]**	

[a]P-values were calculated using Pearson's chi-squared test, comparing the distribution of patients prescribed antibiotics or generic name antibiotic prescriptions, between the two hospitals. [b]P-values were calculated using Student's t test, comparing the mean days of hospital stay or antibiotic treatment duration between the two hospitals. Significant P values are shown in bold type. AB, antibiotic; GE, gastroenteritis; NTH, non-teaching hospital; RTI, respiratory tract infections; TH, teaching hospital.

Table 9. DDDs prescribed per 1000 patients, accounting for 90% of the entire antibiotic prescribing within specific diagnosis groups at paediatric departments in the NTH and TH in Ujjain, India (Paper II)

Antibiotic groups and subgroups	GE		RTIs		Enteric fever		Viral fever		Fever	
	TH	**NTH**	**TH**	**NTH**	**TH**	**NTH**	**TH**	**NTH**	**TH**	**NTH**
ANTIBACTERIALS FOR SYSTEMIC USE: J01	886	1150	2301	2008	4746	4388	1019	1977	1832	2890
β-lactam AB, penicillin: J01C	126	33	747	522		225	117	63	447	73
Extended-spectrum penicillins: J01CA	39	21	130	145	-	84	74	36	112	-
Combination of penicillins including β-lactamase AB: J01CR	87	11	617	376	-	142	43	27	335	73
Other β-lactams: J01D	437	402	849	477	4295	1536	736	1358	1156	1887
2nd generation cephalosporins: J01DC	-	-	-	125	-	211	-	605	-	1179
3rd generation cephalosporins: J01DD	437	400	849	348	4295	1283	736	730	1156	707
Aminoglycosides: J01G	237	160	463	58	300	94	155	21	228	45
Other aminoglycosides: J01GB	237	160	463	58	300	94	155	21	228	45
Quinolones: J01M	46	57	39	79	117	23	8	22	-	-
Fluoroquinolones: J01MA	46	57	39	79	117	23	8	22	-	-
Combination of ABs: J01R	-	442	105	861	-	2465	-	506	-	886

AB, antibiotic; DDD, defined daily doses; GE, gastroenteritis; NTH, non-teaching hospital; RTI, respiratory tract infections; TH, teaching hospital.

Of all 243,790 patients admitted to the NTH and TH from 2008 to 2017, 3,766 (1.5%) were registered with cellulitis, IE, peritonitis, pneumonia, septic arthritis, epiglottitis, sepsis, meningitis or pyelonephritis (**Paper III**). Of these patients, 92% of those at the NTH and 89% of those at the TH were prescribed at least one antibiotic (Table 4). The most common diagnoses among patients with severe infections admitted to NTH and TH during the study period were cellulitis, peritonitis, pneumonia and sepsis, which comprised 88% of the included patients at NTH and 93% of the included patients at the TH (**Paper III**). Antibiotic groups comprising at least 75% of the total antibiotic prescribing among these diagnoses at each

hospital are presented in Appendix II. Antibiotics of the J01CR groups (combinations of penicillins including β-lactamase inhibitors) and J01D (β-lactam antibiotics) were among the most commonly prescribed antibiotics among all diagnosis groups at both hospitals. The total antibiotic prescribing over time is presented in Figure 3 and Table 10. Antibiotics included in the antibiotic groups are presented in Appendix II.

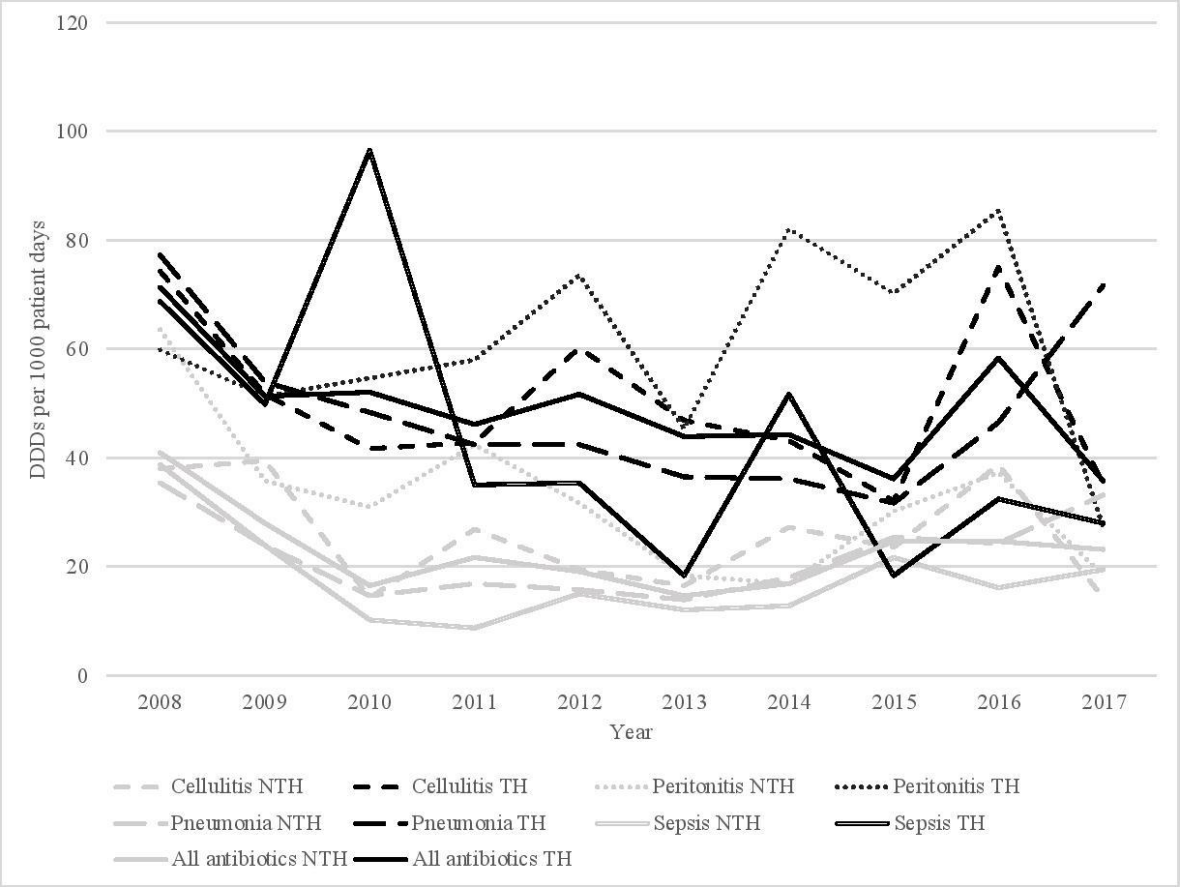

Figure 3. Prescribing of antibiotics among patients with severe infections in the NTH and the TH in Ujjain, India, from 2008 to 2017 (Paper III). Prescribing is quantified and presented in DDDs per 1000 patients (y-axis) each year (x-axis) for selected infectious diseases for each hospital. Trends for each slope (see Table 10), indicating an overall positive or negative trend of antibiotic prescribing (measured in DDDs per 1000 patients), over the study period were obtained by linear regression analyses. DDD, defined daily dosis; NTH, non-teaching hospital; TH, teaching hospital.

Table 10. Description of trends in antibiotic prescribing among patients with severe infections at the NTH and TH in Ujjain, India during 2008–2017 (Paper III)

	NTH	TH
Antibiotic prescribing among specific diagnoses, t[a] (P value[b])		
All antibiotics	**13.84 (<0.01)**	1.82 (0.07)
Cellulitis	**5.72 (<0.01)**	**6.52 (<0.01)**
Peritonitis	**14.59 (<0.01)**	**18.52 (<0.01)**
Pneumonia	**4.87 (<0.01)**	**7.30 (<0.01)**
Sepsis	**2.18 (0.03)**	**-21.91 (<0.01)**
Antibiotic prescribing among all included patients, t[a] (P value[b])		
Access antibiotics	**11.52 (<0.01)**	1.78 (<0.07)
Watch antibiotics	**9.63 (<0.01)**	**6.49 (<0.01)**
Reserve antibiotics	-0.76 (0.45)	**2.54 (<0.01)**
FDCs of antibiotics	**14.28 (<0.01)**	**7.31 (<0.01)**
Antibiotic prescribing among sepsis patients, t[a] (P value[b])		
Access antibiotics	1.49 (0.14)	**-16.89 (<0.01)**
Watch antibiotics	**3.02 (<0.01)**	**-11.38 (<0.01)**
Reserve antibiotics	**-9.32 (<0.01)**	Too few prescriptions
FDCs of antibiotics	**3.78 (<0.01)**	**-9.93 (<0.01)**

[a]t values were obtained by linear regression and indicate a positive or negative trend of antibiotic prescribing (measured in DDDs per 1000 patients), over the study period. A positive t shows a positive trend and a negative t shows a negative trend of prescribing. [b]P value were calculated using linear regression analysis, evaluating the difference (increase or decrease indicated by the slope) in antibiotic prescribing over the 10-year period. Statistically significant P values indicate significant trends and are shown in bold font. DDD, defined daily dose; FDC, fixed-dose combination; NTH, non-teaching hospital; TH, teaching hospital.

At the NTH, *access* antibiotics comprised 40% of the total antibiotic prescribing among patients with severe infections during 2008-2017; *watch* antibiotics 40%, reserve antibiotics < 1% and FDCs of antibiotics 18%. At the TH, *access* antibiotics comprised 61% of the total antibiotic prescribing among patients with severe infections in 2008-2017, *watch* antibiotics 29%, reserve antibiotics <1% and FDCs of antibiotics 8%. The prescribing of *access*, *watch* and FDCs of antibiotics increased from 2008 to 2017 at the NTH ($P < 0.05$) while at the TH, prescribing of *watch*, reserve and FDCs of antibiotics increased from 2008 to 2017 ($P < 0.05$ respectively). The prescribing of *access*, *watch, reserve* and FDCs of antibiotics are presented in Figure 4 and Table 10.

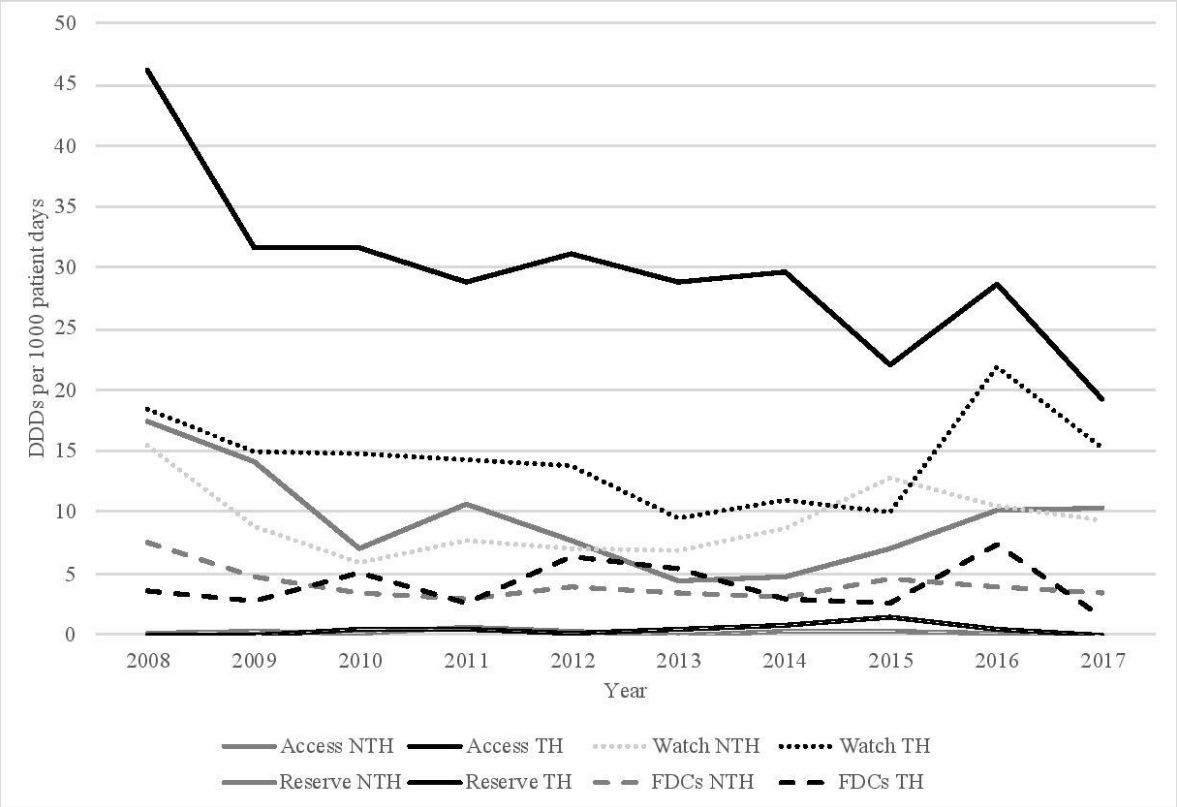

Figure 4. Prescribing of antibiotics categorized by *access, watch, reserve* and FDCs of antibiotics among patients with severe infections in the NTH and the TH in Ujjain, India, from 2008–2017 (Paper III). Prescribing is quantified and presented in DDDs per 1000 patients (y-axis) each year (x-axis) of the total prescribing rate of each antibiotic category (*access, watch, reserve* and FDCs) for each hospital. Trends for each slope, indicating an overall positive or negative trend of antibiotic prescribing (measured in DDDs per 1000 patients), over the study period were obtained by linear regression analysis. DDD, defined daily dose; FDC, fixed-dose combinations; NTH, non-teaching hospital; TH, teaching hospital.

4.3 DIAGNOSTICS

For **Paper IV**, data on 492 patients with definite IE (according to modified Duke criteria for IE) were obtained from the SRIE (64, 67). Clinical characteristics and predisposing factors of the included patients are presented in Table 11.

Table 11. Descriptive data of patients with IE admitted to the Karolinska University Hospital in Stockholm, from 2008 to 2017 (Paper IV)

All patients, n (%)	492 (100)
Women, n (%)	161 (33)
Men, n (%)	331 (67)
Age, mean (± SD); medians (25th and 75th percentiles)	57.1 (18.4); 57 (44.5, 72)
Predisposing factors	
History of IV drug abuse, n (%)	156 (32)
Bicuspid aortic valve, n (%)	18 (4)
Prosthetic valve, n (%)	92 (19)
Cardiovascular implantable electronic device, n (%)	50 (10)
Rheumatic heart disease, n (%)	2 (0)
Congenital heart disease, n (%)	7 (1)
History of IE, n (%)	78 (16)
Known valvular disease, n (%)	75 (15)
Heart failure before or during IE treatment, n (%)	60 (12)
Clinical characteristics	
Fever, n (%)	432 (88)
Vascular phenomena, n (%)	210 (43)
New heart murmur, n (%)	77 (16)

IE, infective endocarditis; IV, intravenous; SD, standard deviation.

There was no significant difference in mean age between male (56.8 years) and female patients (57.8, P = 0.58). The most common bacterial pathogens among the patients with IE were *S. aureus*, (239 patients, 49%), viridans group streptococci (102 patients, 21%) and *E. faecalis* (50 patients, 10%, Appendix III), and no gender differences in aetiology were found. In total, 435 (88%) of the patients underwent TEE, 270 (56%) underwent both TTE and TEE and 57 (12%) underwent TTE alone. In total, 409 patients (83%) had one IE manifestation and 83 patients (17%) had more than one detected by ECHO. The distribution of IE manifestations detected by ECHO and bacterial aetiologies are presented in Appendix III. Bacterial aetiologies among patients with IE with IV drug abuse, prosthetic heart valves, prevalence of more than one IE manifestation, surgical treatment for IE, and patients with IE who died during hospital stay are presented in Appendix IV. The IE manifestations detected by ECHO among patients with IE with IV drug abuse, surgical treatment for IE and patients with IE that died during hospital stay are presented in Appendix V.

Of the 239 patients with *S. aureus* aetiology, 122 (51%) had a history of IV drug abuse and 117 (49%) did not. There was an association between *S. aureus* infection and tricuspid valve vegetation among patients with a history of IV drug abuse (OR 4.75; 95% CI 1.92–12.52; P < 0.01) but not among patients who did not have IV drug abuse (OR 1.96, 95% CI 0.74–5.16; P = 0.12).

Among patients with native aortic valves, the presence of aortic valve vegetation was significantly associated with findings of *E. faecalis* (OR 3.64; 95% CI 1.65–8.24; P < 0.01) and closely significantly associated with coagulase-negative staphylococci (CoNS) (OR 2.83; 95% CI 0.91–9.14; P = 0.04). However, there were no significant associations between aortic valve vegetation and *E. faecalis* (OR 0.67; 95% CI 0.20–2.36; P = 0.47) or CoNS (OR 3.89; 95% CI 0.46–180.98; P = 0.18) respectively, among patients with prosthetic aortic valves.

In our cohort, 92 patients (19%) had prosthetic heart valves, and 7 (8%) of these patients had two prosthetic heart valves. Patients with prosthetic heart valves were more likely to have *E. faecalis*-linked IE (Appendix IV). There was an association between the incidence of prosthetic heart valves and perivalvular abscesses (no specific locations were registered) (OR 17.64; 95% CI 6.89–50.34; P < 0.01). The described associations refer to the whole cohort of patients with prosthesis-associated IE in our study without differentiation between early- and late-onset IE, because this was not possible.

In total, 139 (28%) patients were treated surgically. There was no difference in age between patients who did or did not undergo surgical treatment for IE (surgery mean age was 55 years, non-surgery mean age was 57.9 years; P = 0.12). There was no difference in surgical treatment between patients with prosthetic valve IE (n = 26) and patients with native valve IE (n = 113), (OR 1.00; 95% CI 0.58–1.69; P = 1.00). Patients with a history of IV drug abuse were treated less frequently with cardiac surgery (26 patients, 17%, OR 0.39; 95% CI 0.23–0.65; P < 0.01). There were no significant differences in in-hospital mortality between surgical (n = 12, 2%) and non-surgical treatment (n = 21, 4%; OR 1.49; 95% CI 0.65–3.29; P = 0.28). In patients presenting with an aortic root abscess, there was no significant association between surgical

treatment and in-hospital mortality (OR 0.75; 95% CI 0.07–11.07; P = 1.00), or between non-surgical treatment and death (OR 1.33; 95% CI 0.09–4.58; P = 1.00).

The overall in-hospital mortality was 7% (33 patients). There was an association between presence of perivalvular abscess and in-hospital mortality (n = 6; OR 4.21; 95% CI 1.29–11.81; P < 0.01, Appendix V). Among patients with *S. aureus* aetiology, in-hospital mortality was more common among left-sided valve IE (n = 14, 6%), compared with right-sided valve IE (n = 1, <1%; OR 9.63; 95% CI 1.40–412.09; P = 0.01). In addition, patients with *S. aureus* IE and left-sided valve IE were significantly older than those with *S. aureus* IE and right-sided valve IE (mean age right-sided valve IE: 38.2 years, mean age left-sided valve IE: 60.3 years; P < 0.01). Prosthetic valve IE was not associated with in-hospital mortality (OR 1.43; 95% CI 0.54–3.41; P = 0.40). There was a significant association between more than one IE manifestation on ECHO and in-hospital mortality (OR 2.69; 95% CI 1.12–6.10; P < 0.01).

Men were more likely to have aortic valve vegetation than women (OR 1.77; 95% CI 1.16–2.71; P = 0.01). Moreover, bicuspid aortic valves were marginally significantly more common in men than in women (OR 4.04; 95% CI 0.93–36.56; P = 0.05). There were no differences between men and women in terms of IV drug abuse (OR 0.92; 95% CI 0.60–1.41; P = 0.69), presence of prosthetic heart valves (OR 1.07; 95% CI 0.64–1.81; P = 0.79), in-hospital mortality (OR 0.84; 95% CI 0.38–1.93; *P* = 0.64), or surgical treatment for IE (OR 1.35; 95% CI 0.86–2.14; P = 0.17).

5 DISCUSSION

The burden of antibiotic resistance is known to be high in India (109). To combat this trend, the WHO recommends mapping and analysing antibiotic prescribing to identify areas in need of improvements (10). This thesis presents antibiotic prescribing practices in two private sector hospitals in the Ujjain district in Madhya Pradesh, India. When data were collected for the papers included here, access to diagnostic methods was limited and antibiotic prescribing guidelines were not implemented at either of the hospitals. Analysis of the antibiotic prescribing practices at the hospitals shows high and increasing use of antibiotics, even among patients with no apparent relevant indications for such treatment. Further, high and increasing use of broad-spectrum antibiotics as well as FDCs of antibiotics were seen at both hospitals. These findings indicate that there is a need for improvements in antibiotic prescribing practices and management of infectious diseases at these hospitals. This thesis also presents significant associations between bacterial aetiology and infectious manifestations detected by ECHO in patients with IE. ECHO is an important part of the routine management of IE in high-income settings as it is an effective method to identify it and conduct follow-ups. As it is a relatively easily implemented diagnostic method in resource-limited settings, the findings of this thesis could possibly be useful in the management of IE in such settings.

5.1 AREAS OF POTENTIAL IMPROVEMENT IN ANTIBIOTIC PRESCRIBING PRACTICES IN A RESOURCE-LIMITED SETTING

5.1.1 Antibiotics were sometimes prescribed without proper indication

At medical ICUs at the two hospitals in Ujjain, most of the admitted patients were prescribed antibiotics (NTH 71% and TH 70%) **(I)**. Despite the high antibiotic prescribing rates, only 12% of the patients at the medical ICUs at the NTH and 14% at the TH were registered with a diagnosis of infection. Even though infectious diseases were known to be common among those seeking health care in a previous study in Ujjain (110), most of the patients at the medical ICUs who were prescribed antibiotics had no diagnosis of infection registered (73% of the patients prescribed antibiotics at the NTH and 69% of the patients prescribed antibiotics at the TH had no diagnoses of infections or presumed symptoms of infection) **(I)**. These findings are supported by a study conducted in an ICU of an Indian TH where 55% of the patients prescribed antibiotics had no reported bacterial infections (111). Antibiotics were also commonly prescribed as presumed symptomatic treatments at medical ICUs at both hospitals (at NTH, 93% of the patients with presumed symptoms of infection recieved antibiotics and 85% at the TH) **(I)**. In line with our results, high prescribing of antibiotics has been described in previous studies from medical ICUs and mixed ICUs in India, covering 80–95% of the patients (112-114).

Antibiotics were commonly prescribed among patients at the paediatric departments at the NTH (84% of patients). However, a lower percentage (44%) of patients at the paediatric departments at the TH were prescribed antibiotics **(II)**. Paediatric patients with unspecified fever were more commonly prescribed antibiotics at the NTH (88% of the paediatric patients

with unspecified fever at the NTH and 47% at the TH), although antimicrobial agents are adviced not to be prescribed empirically to children with unspecified fever (115). In addition, the antibiotic prescribing rates among patients with acute gastroenteritis and viral fever, diagnoses for which antibiotics are not considered as proper treatment (54, 110, 116), were higher at the NTH compared with the TH; however, most of the patients with acute gastroenteritis and viral fever were prescribed antibiotics at the TH. An Indian study indicates that viral infections are the most common cause of fever in Indian children, and suggest that paediatric patients presenting with fever should be evaluated with clinical examination and relevant investigations but not treated with antibiotics (115). Antibiotics could possibly have been prescribed for superimposed bacterial infection among paediatric patients with viral fever at the NTH and TH.

At both the NTH and the TH, samples were seldom sent for microbiological analysis, which suggests that most of the antibiotics were empirically prescribed on suspicion of infection (**Papers I–III**). However, at the NTH and the TH, laboratories for biochemical and microbiological confirmation of bacterial infections were easily available and free of charge, but despite that, underutilized at both hospitals. Underutilization of laboratory facilities has also been described in a study from a medicine department in a tertiary care, non-profit TH in Moradabad, India, where antibiotics accounted for 59% of all medicine prescriptions but only 3% out of 180 patients underwent microbiological culture investigations (117). The reasons for underutilization of laboratory facilities need to be explored further. The lack of diagnostic investigations may have contributed to unnecessary prescribing of antibiotics or underprescribing of antibiotics in cases where an infection was present but not diagnosed. Since no diagnostic methods were routinely used either at the NTH nor at the TH, verification of the site of infections and infectious aetiologies were not possible to assess. Therefore, it was impossible to evaluate whether the antibiotics were also prescribed rationally or not. The *access* (antibiotics recommended as first-line treatments), *watch* (antibiotics recommended as second-line treatments) and *reserve* (antibiotics recommended as third-line treatments) categorization of antibiotics provides a practical guide for proper antibiotic prescribing and can provide a basis for the development of local prescribing guidelines (107). In the TH, prescribing of *watch*, reserve and FDCs of antibiotics increased over 2008-2017 while at the NTH, prescribing of *access*, *watch* and FDCs of antibiotics increased among patients with severe infections (**Paper III**). Implementation of antibiotic prescribing guidelines might improve the prescribing of recommended antibiotics. Implementation of guidelines and diagnostic methods would enable more targeted prescribing to limit the unnecessary use of *watch* and *reserve* antibiotics.

5.1.2 Adherence to antibiotic prescribing guidelines varied between hospitals

The WHO has published international prescribing guidelines for antibiotic treatment (WHOLEM) and there are national lists of essential medicines in India including recommendations of antibiotics for both adult (NLEMI) and paediatric patients (IAP-LEM) (104, 106). Among antibiotic prescriptions for patients in medical ICUs, compliance with the

WHOLEM and NLEMI was higher at the TH compared with the NTH (prescriptions adherent to the WHOLEM, NTH 47% vs. TH 52%; P < 0.001; those to the NLEMI, NTH 53% vs. TH 70%; P < 0.001, **Paper I**). Our results show lower adherence to guidelines compared with a study of a medical ICU in Gujarat, where 76% of the antibiotic prescriptions were adherent to the local antibiotic policy of the hospital (112). However, our results are more similar to another Indian study, also from Madhya Pradesh, that compared prescriptions from general practitioners in rural and urban areas, where 63% of the prescriptions in the urban area were adherent to lists of essential medicines compared with 75% of the prescriptions in the rural area (118) and to a study comparing prescriptions from general practitioners in rural and urban areas of Tamil Nadu showed that 35% of the prescriptions in the rural area and 40% in the urban area were adherent to the list of essential medicines (119).

Among the paediatric patients, less than 40% of the antibiotic prescriptions were adherent to the IAP-LEM. However, the adherence to the IAP-LEM was significantly higher at the TH compared with the NTH (prescriptions adherent to the IAP-LEM: NTH 24% vs. TH 37%; P < 0.001) (**Paper II**). At both hospitals, most paediatric patients with enteric fever were prescribed third-generation cephalosporins by the parenteral route although this is only recommended for patients with complicated or multi-drug-resistant enteric fever (120). Antibiotic treatment durations for paediatric patients with RTIs were a mean of 4.3 days at the NTH and 6.4 days at the TH. Antibiotic prescribing guidelines recommend 5–7 days of antibiotic treatment for children with mild or moderate forms of community-acquired pneumonia and up to 14 days for severe pneumonia, which suggests that the TH was more adherent to the guidelines (121).

Overall, durations of hospital stay and antibiotic treatment durations were significantly longer at the TH **(Papers I-III)**. The shorter durations of hospital stay at the NTH, could possibly be explained by economic factors. At the NTH, patients had to pay for their stay and treatment in hospital, which might have contributed to shorter durations of stay than in the TH, where patients did not pay and possibly could stay longer in hospital. Further, the physicians at the NTH were paid for the number of patients they admitted to the hospital, which might have contributed to shorter durations of hospital stay to be able to admit new patients. Two studies support our results, one from a medical ICU in a rural hospital in Gujarat, India–where patients had to pay for their treatment–showing a similar duration of hospital stay as the NTH (mean 3.0 days) (112), and another study from an ICU in a TH in Northern India that reported a mean duration of hospital stay of 5.7 days for medical patients, which is more similar to the TH (113).

According to guidelines, antibiotics should be prescribed by generic names rather than trade names, to ensure good quality antibiotics, give flexibility to dispense from available formulations and promote antibiotic access in LMICs (122, 123). Prescribing of generic name antibiotics was higher at the TH compared with the NTH (**Papers I and II**). High prescribing of trade names and low prescribing of generic name antibiotics have been described previously. A study from a tertiary care hospital in Uttar Pradesh showed that only 1.8% of the antibiotics were prescribed by generic name (117). The management of the TH controlled the purchase and supply of medicines at the hospital, possibly with the aim if obtaining good quality and

cost-effective medicines, and thus contributing to the higher prescription rate of generic name antibiotics. The high prescribing of trade name antibiotics at the NTH might be explained by the presence of medical representatives from pharmaceutical companies at the NTH (but not at the TH), which are known to put pressure on and influence the prescribers (28, 124-126). A study from the USA reported that prescribers exposed to representatives from pharmaceutical companies were more likely to prescribe medicines by trade name rather than generic name (125).

There are few studies analysing why physicians do not follow recommendations for antibiotic treatment but one factor that is known to contribute to the prescribing of non-recommended antibiotic treatments is the limited availability of guidelines and lists of essential medicines, or limited knowledge about existing guidelines (126-128). When the data were being collected for this thesis, antibiotic prescribing guidelines were not implemented at either of the hospitals, which could explain the relatively low adherence to international and national guidelines. The higher compliance with the WHOLEM and the NLEMI or IAP-LEM at the TH could possibly be explained by the controlled purchase and supply of medicines, both for quality and economic reasons, because those included in lists of essential medicines are less expensive than, for example, the FDCs of antibiotics that were more commonly used at the NTH. Further, the feeling of responsibility among physicians participating in clinical education of medical students could have contributed to a more proper prescribing pattern at the TH.

5.1.3 Prescribing of broad-spectrum antibiotics and FDCs of antibiotics was high and increasing

Among patients in medical ICUs, β-lactam antibiotics (J01C and J01D), especially combinations of penicillins including β-lactamase antibiotics (J01CR) and third-generation cephalosporins (J01DD), quinolones (J01M), especially fluoroquinolones (J01MA) and imidazole derivatives (J01XD) were commonly prescribed at the TH. At the NTH, β-lactam antibiotics (J01C and J01D), especially combinations of penicillins including β-lactamase antibiotics (J01CR) and third-generation cephalosporins (J01DD) and FDCs of antibiotics were commonly prescribed among such patients (**Paper I**). Prescribing practices at the TH were similar to that in a medical ICU in a teaching hospital in South India, where third-generation cephalosporins (ceftriaxone), combinations of penicillins including β-lactamase antibiotics (piperacillin with tazobactam) and imidazole derivatives (metronidazole) were the most commonly prescribed antibiotics during the study period (1 January 2014 to 31 December 2014) (114). Further studies that support our results were from a rurally located community hospital in Chhattisgarh where penicillins, aminoglycosides, quinolones and imidazole derivatives were commonly prescribed (129) and a public sector tertiary care TH in a rural area in Chandigarh, where cephalosporins, aminoglycosides and metronidazole comprised approximately 70% of the antibiotics prescribed (130). At an ICU in a public sector TH in Nagpur, 32% of the in-patients were prescribed third-generation cephalosporins (111) and in an ICU in a NTH in Maharashtra where patients were charged for medical treatment, penicillins including β-lactamase antibiotics and third-generation cephalosporins were commonly

prescribed, which supports the findings in our study (131). Lack of time, inadequate knowledge and the physicians' perception of expectations from the patients, diagnostic uncertainty and fear of negative outcomes are factors that might contribute to prescribing broad-spectrum antibiotics (126-128).

In the paediatric departments at the NTH, FDCs of antibiotics were most commonly prescribed among paediatric patients with acute gastroenteritis, RTIs and enteric fever while among paediatric patients with viral and unspecified fever, second- and third-generation cephalosporins were more commonly prescribed. At the TH, third generation cephalosporins were the most commonly prescribed antibiotics among all diagnosis groups of paediatric patients (**Paper II**).

Prescribing FDCs of antibiotics among patients with severe infectious diseases increased both in the NTH and TH during 2008–2017 (**Paper III**). Prescribing FDCs of antibiotics is not recommended as they have been shown to drive antibiotic resistance, contributing to further unnecessary use (48). A factor that is known to contribute to prescribing FDCs of antibiotics is pressure from pharmaceutical companies (126-128). This factor is known to influence physicians' prescribing practices in India (124). The higher rate of prescribing FDCs of antibiotics at the NTH could possibly be explained by the presence of representatives from pharmaceutical companies who commonly promote prescribing FDCs of antibiotics (132, 133). The prohibition of representatives from pharmaceutical companies at the TH might have contributed to less such prescribing, as mainly generic medicines are procured by the TH's management.

5.2 IMPROVED MANAGEMENT OF INFECTIOUS DISEASES USING DIAGNOSTICS

Diagnostics are essential to verify infections and their aetiology, to guide treatment regimens and to evaluate effects of interventions (134). Diagnostics are also important to contain the burden of infectious diseases in LMICs (135, 136). A panel of experts in global health has ranked modified molecular technologies for affordable, simple diagnostics of infectious diseases as the top-rated biotechnology for improving health in LMICs (134). Historically, diagnostic methods have been expensive and resource-demanding, making them not easily available in LMICs (134, 135). Although affordable diagnostics have the potential to reduce mortality in infectious diseases in LMICs, they have not been implemented (135, 136). So-called point-of-care diagnostics are less dependent on contextual factors or explicitly trained staff and are specifically designed to be used in resource-limited settings (137). The recent development of affordable ultrasound machines of good quality has enabled the implementation of so-called point-of-care ultrasonography, which provides benefits for health-care settings with limited access to advanced diagnostic methods (138). ECHO has also been evaluated to have high clinical utility in resource-limited settings (139). Thus, the cardiac manifestations of IE can be detected by ECHO with some basic training of health-care personnel (140).

At the KUH in Stockholm, both ECHO and microbiological analysis are parts of the routine management of patients with suspected or defined IE and there is a registry covering most of the patients with IE admitted to the hospital since 2008 (102). **Paper IV** aimed to assess any associations between IE manifestations detected by ECHO and bacterial aetiology in affected patients. The findings could possibly have importance in the management of such patients in a setting where ECHO is available but access to laboratory facilities for culture tests is limited or absent.

5.2.1 How can IE be managed by ECHO – an example from a high-income setting

In **Paper IV**, several associations between specific IE manifestations detected by ECHO and certain bacterial species were found; between native aortic valve vegetation and *E. faecalis*; between mitral valve vegetation and group B streptococci and viridans group streptococci; between tricuspid valve vegetation and *S. aureus* among patients with IV drug abuse; between perivalvular abscesses and CoNS; and between CIED-associated IE and HACEK (combination of *Haemophilus* species, *Aggregatibacter* species, *Cardiobacterium hominis*, *Eikenella corrodens* and *Kingella Kingae*) and CoNS. Consistent with our results, it has been shown that CoNS caused perivalvular extension of infections in patients with IE (89). The association between tricuspid valve IE and *S. aureus* infection has been reported in a previous study (88).

The most common IE manifestations were vegetations in the mitral (40%), aortic (39%) and tricuspid valves (22%), reminiscent of a study of 68 autopsies of patients with IE reporting that 35% of patients had mitral valve vegetation, 26% had aortic valve vegetation and 5% had tricuspid valve vegetation (141). The high prevalence of tricuspid valve vegetation in our study might be explained by the high percentage of patients with a history of IV drug abuse (32%). Further supporting our findings, a multicentre study of 1,055 patients with IE in Europe and the USA showed that mitral valve followed the aortic valve vegetations were the most common ECHO manifestations (142). TTE is a good method for visualization of native valve IE, with 96% sensitivity, which is equal to the sensitivity of TEE for native valve IE (64, 143, 144). However, in patients with prosthetic heart valves, IE manifestations are more difficult to detect with TTE because of shading from the prosthesis or chordae tendinae, and the relatively low resolution of TTE giving a sensitivity of 70% compared with 92% for TEE. Therefore, TEE is highly recommended for patients with prosthetic heart valves (64, 144, 145). Further, ECHO findings might not differ between patients with IE undergoing ECHO early (<2 days) or late (≥2 days) after the initiation of antibiotic treatment (90).

The most common bacterial species were *S. aureus* (49%) and viridans group streptococci (21%). Previous studies of patients with IE in industrialized countries also showed that *S. aureus* is the most common bacterium causing IE (90, 146-148) and that the prevalence rates of viridans group streptococci are high and increasing (145, 149). *S. aureus* has been described as being linked to higher mortality rates among patients with IE, compared with other bacterial species (90, 146-148). In our study, *S. aureus* was not associated with higher in-hospital mortality; however, 51% of all patients with *S. aureus* aetiology had a history of IV drug abuse.

Such patients are known to be commonly affected with *S. aureus* and to acquire right-sided rather than left-sided valve vegetations (150-152). Among the patients with *S. aureus*-related IE, patients with right-sided valve vegetations were younger and had lower in-hospital mortality compared with the patients with left-sided valve vegetations. The overall in-hospital mortality in our study (7%) was lower than the previously reported rates of 15–20% (153, 154), but similar to a Swedish study showing a 30-day crude mortality rate of 10.4% (146). The relatively low age of the included patients with IE (mean 57.1 years) and high prevalence of right-sided IV drug abuse-associated IE might have contributed to the low in-hospital mortality in our study. Perivalvular abscess was associated with in-hospital mortality, a finding supported by Lauridsen et al. who reported that perivalvular abscess predicted 1-year mortality in patients with left-sided native valve *S. aureus*-linked IE (155).

Patients with IE with a history of IV drug abuse were less commonly treated with cardiac surgery compared with those with no such history, which might explain the relatively low rate of patients who underwent surgical treatment in our study (28%). Surgical treatment was more common among patients with aortic valve vegetation, CIED-associated endocarditis or a perivalvular abscess. In our study, there was an association between prosthetic valve IE and an aetiology of *E. faecalis* and a negative association between prosthetic valve IE and *S. aureus*. These findings differ from previous studies showing that *S. aureus* and CoNS were common among patients with prosthetic valve IE (149). In our cohort, there were more male than female patients included (ratio approximately 2:1), a finding that is supported by earlier studies of patients with IE (156, 157). Male patients with IE were more likely to have aortic valve vegetations than female, which is partly consistent with previous studies (157, 158). In-hospital mortality and surgical treatment were equal among male and female patients, although there are previous studies reporting higher mortality rates as well as a lower rate of surgical treatment among female patients (88).

5.2.2 Can ECHO be implemented for improved management of IE in resource-limited settings?

The results from **Paper IV** suggest the possibility of predicting bacterial aetiology with ECHO, as certain IE manifestations detected by ECHO are associated with specific bacterial aetiology in patients with IE. This might have importance in a setting where culture tests are scarce or absent, but ECHO might be possible to implement. According to the modified Duke criteria, the diagnosis of definite IE can be stated not only with the two major criteria fulfilled (blood culture and imaging both positive for IE) but also if one major and three minor criteria, or five minor criteria are present (Table 1) (64, 67). Consequently, the diagnosis of definite IE could be established using ECHO and clinical evaluation of the patients, also if microbiological culture investigations are absent or limited, which could be valuable in resource-limited health-care facilities.

Significant associations were found between ECHO-detected IE manifestations and specific bacterial species among Swedish patients with IE, suggesting that the prediction of bacterial aetiology with ECHO might be possible in some cases. To ensure the findings can be applicable

to populations other than this cohort from Stockholm, it will be important to compare clinical characteristics between the patients included in our cohort, and patients in other populations. The management and causes of IE, as well as patient characteristics, might also differ between high-income countries and LMICs. Comparing the cohort of patients with IE from Stockholm with previously described characteristics of patients with IE in India shows that there are both differences and similarities. Table 12 shows a summary of the findings from four Indian studies as well as from **Paper IV**.

Table 12. Clinical characteristics of patients with IE in four studies from tertiary care hospitals in India, and comparisons with Paper IV

	Choudhury et al.[a] Chandigarh, Punjab	**Subbaraju et al.**[b] Manipal, Karnataka	**Soman et al.**[c] Mumbai, Maharashtra	**RM et al.**[d,] Chennai, Tamil Nadu	**Paper IV,** Stockholm, Sweden
Study period	1981–1991	2007–2013	2007–2015	2010–2015	2008–2017
Number of patients, n	186	139	53	120	492
Definite IE/possible IE, n (%); n (%)	186 (100); 0 (0)	95 (68); 44 (32)	44 (83); 9 (17)	120 (100); 0 (0)	492 (100); 0 (0)
Age, mean years	25	48	>77% older than 40 years	53	57
Sex ratio, male: female	-	2.2:1	-	2.5:1	2:1
Prosthetic valves, n (%)	2 (1)	5 (4)	15 (28)	17 (14)	92 (19)
RHD, n (%)	78 (42)	43 (31)	7 (13)	18 (15)	2 (0)
Underlying heart disease, n (%)	GUCH: 33 (17)	135 (97)	29 (55)	90 (62)	322 (65)
IV drug abuse, n (%)	1 (<1)	0 (0)	-	0 (0)	156 (32)
Staphylococci, n (%)	37 (20)	MSSA: 15 (11), CoNS: 3 (2)	4 (8)	11 (9)	SA: 239 (49), CoNS: 24 (5)
Streptococci, n (%)	34 (18)	VGS: 43 (31)	VGS: 14 (26)	19 (16) VGS: 16 (13)	133 (27) VGS: 102 (21)
Enterococci, n (%)	-	18 (13)	9 (17)	16 (13)	52 (11)
Positive ECHO, n (%)	121 (64)	131 (94)	53 (100)	86 (97)	492 (100)
ECHO findings, n (%)	-	MV: 80 (58), AV:27 (19), TV:8 (6), PV: 3 (2)	-	MV: 50 (42), AV:47 (39), TV:7 (6), >1 valve: 16 (13)	MV:195 (40), AV:190 (39), TV:108 (22), >1 valve: 83 (17)
Surgical treatment, n (%)	-	5 (4)	26 (49)	31 (26)	139 (28)
In-hospital mortality, n (%)	47 (25)	24 (17)	11 (21)	12 (10)	33 (7)

Adapted from: [a](159)[b](74)[c](71)[d](70). AV, aortic valve; CoNS, coagulase-negative staphylococci; ECHO, echocardiography; GUCH, grown-up congenital heart disease; IE, infective endocarditis; IV, intravenous; MSSA, methicillin-sensitive S*taphylococcus aureus*; MV, mitral valve; PV, pulmonary valve; RHD, rheumatic heart disease; SA, S*taphylococcus aureus*; TV, tricuspid valve; VGS, viridans group streptococci.

A study from 1992 conducted in Chandigarh, Punjab, of 186 patients admitted between 1981 and 1991 with definite IE, showed that RHD was present in 42% of the patients (159). There seems to be a variation in presence of RHD in patients with IE in India according to three relatively recent studies from tertiary care centres in three Indian cities. Two studies published in 2018, one conducted in Mumbai, including 53 patients for 2007–2015 and one from Chennai, including 145 patients admitted during 2010–2015, showed presence of RHD in 13% and 15% of the patients, respectively (70, 71). A study from Manipal (2007–2013) of 139 patients with IE reported a higher incidence of RHD (31%). Thus, RHD seems to be more common among Indian patients with IE compared with our cohort with only two such patients (<1%, **Paper IV**). In the two more recent Indian studies, the rates of underlying heart diseases were similar to our study (Table 12). The three studies from Mumbai, Chennai and Manipal showed that viridans group streptococci were the most common bacterial strains with similar rates to our **Paper IV** (70, 71, 74). The three studies showed a much lower rate of *S. aureus*-associated IE compared with **Paper IV**, which might be explained by the absence of IV drug-abuse patients in their study populations (<1%) compared with 32% of patients with history of IV drug abuse in our cohort (70). The presence of enterococci seemed to be relatively similar between the Indian studies and those we report in **Paper IV** (Table 12). In the study from Chennai, the rates of mitral and aortic valve vegetations were similar to our study (Chennai, 42% and 39%, vs **Paper IV**, 40% and 39%), but tricuspid valve vegetation seemed to be more common in the results of **Paper IV**, also possibly associated with the higher rate of IV drug abuse (70). The higher rate of history of drug abuse probably contributed to the higher rate of tricuspid valve vegetations in **Paper IV**. Tricuspid valve vegetations in Indian patients were mostly present in patients with CIEDs, immunocompromised patients with central venous catheters, and among patients with congenital heart diseases (160). The rate of surgical treatment was similar in the studies from Mumbai and Chennai, as in **Paper IV**, but it should be noted that the Indian studies were from hospitals with access to cardiac surgery, which is not always the case, so surgery rates may vary. For example, in the study from Manipal, only 4% of patients were surgically treated.

Consequently, ECHO is important for the diagnosis, cardiac evaluation and follow-up of IE. The results from **Paper IV** suggest that ECHO might also have a role in the prediction of a bacterial aetiology for IE, which can be valuable in resource-limited health-care facilities. Ultrasonography has been shown to be possible to implement in health-care facilities with limited resources, because it requires fewer economic and personnel assets than many of the conventional diagnostic methods (80, 84, 140, 161). However, the effects of implementation of ECHO in the management of infectious diseases, such as IE, need to be further evaluated in resource-limited health-care facilities.

5.2.3 Implications for the treatment of IE

Successful treatment of IE relies on effective antibiotic treatment (64). The treatment guidelines for IE published by the European Society of Cardiology and the American Heart Association are mostly based on bacterial aetiology (64, 66). In resource-limited settings, the

bacterial aetiology can remain unknown if access to microbiological analysis is limited or absent. The results of this thesis show that ECHO might have a role in the prediction of bacterial aetiology of IE, but it might not be sufficiently robust to guide bacteria-specific treatment alone.

In health-care settings with access to microbiological analysis, the so-called blood culture-negative IE is a phenomenon where no causative microorganism can be verified by the usual blood culture methods. Most studies report that 5–10% of IE cases are blood culture-negative (66) but this can occur in up to 31% of all cases, which might cause substantial predicaments both for making a correct diagnosis and for treatment (64). Empirical treatments that cover the most common bacterial aetiologies of IE are often required. According to the guidelines, blood culture-negative IE should be treated in consultation with a specialist in infectious diseases, with the focus being to identify a pathogen and to make the most appropriate choice of antibiotic treatment (64, 66). An evaluation of patient-specific factors in relation to epidemiological factors is recommended. The epidemiological factors that should be taken into consideration are: any history of prior infections; risk factors or predisposing factors for IE; prior exposure to antibiotics; extracardiac sites of infection; and the clinical course and severity of the current infection (66). Evaluation of clinical and additional laboratory data often enables revision of empirical treatments to a more targeted treatment regimen (66). In addition to its diagnostic value, ECHO can be used for evaluation of the severity of valve infection and cardiac complications, in decision-making regarding the need for surgery, in the evaluation of treatment, and in follow-up (64, 66).

According to our findings in **Paper IV**, ECHO could have a role in clinical evaluation to guide the management and treatment of IE, providing knowledge regarding how certain bacteria manifest in this disorder. To highlight their locations and manifestations, associations with risk factors or predisposing factors for IE, and their associations with certain outcomes, the pattern of different bacteria might be of clinical value for management and treatment. This could be useful also in resource-limited settings, where ECHO could possibly contribute to better management of patients with IE. According to the results from **Paper III**, only 9 patients out of 243,774 admitted to the NTH and TH between 2008 and 2017, were registered with IE, suggesting underdiagnosing of IE at the two hospitals in Ujjain. Implementing ECHO in such settings could contribute to the diagnose, management, treatment and follow-up in patients with IE.

5.3 METHODOLOGICAL CONSIDERATIONS

5.3.1 Strengths

A strength of **Papers I-III** was the prospective and consecutive mode of data collection. The same method for data collection was used at both hospitals, which enabled good comparability of data. At both hospitals, nurses were trained continuously in data collection to ensure its quality and to involve new staff in the collection of data quickly. Another strength was the computerization of records with a possibility to extract data to follow prescribing patterns. Antibiotic prescribing was measured using the internationally accepted ATC/DDD

methodology. Measuring antibiotic prescribing with DDDs enables comparisons between different health-care facilities and comparisons of prescribing over time. In combination with data on diagnoses or indications for prescribing, this methodology can also be used to assess the rationality of prescribing. It should be noted that the DDD methodology is based on medicine use in adult patients, and not in paediatric patients. However, DDDs can also be used to compare antibiotic prescribing for children, between different health-care facilities, and for prescribing over time.

In **Paper IV**, a strength was the information on comorbidities and underlying heart diseases provided by the SRIE. Comorbidities and underlying heart diseases, such as degenerative structural heart disease, or any history of IV drug abuse and RHD, can contribute considerably to aspects of bacterial aetiology, manifestations and mortality in patients with IE. The SRIE includes information about several comorbidities and underlying heart diseases such as RHD, history of IV drug abuse and congenital heart disease, which enables comparisons of the results from studies in other populations of patients with IE.

5.3.2 Limitations

Although the data for **Papers I-III** were collected in a controlled manner, there was a risk of missing data, because data collection and entry were both manual. The registered diagnoses were not confirmed by culture results, unless ordered by the consultant in charge or in any other way. Consequently, most of the diagnoses were based on clinical suspicions and some patients might have been incorrectly diagnosed. Absence of culture results makes it difficult to assess whether the antibiotics were prescribed rationally. However, by analysing compliance with existing guidelines on empirical prescribing for certain infectious diagnoses as well as analysing compliance with the WHO's antibiotic categories of *access*, *watch* and *reserve*, the appropriateness of antibiotic prescribing practices in the NTH and TH could be discussed. Another limitation was the absence of medical records and documentation of previous medical history for the patients included in **Papers I-III**. Because there were no medical records or documentation available, no predisposing factors among the patients could be evaluated.

For **Paper IV**, the SRIE included the locations of IE manifestations and information about known valvular disease prior to a diagnosis, but no detailed information about the size or number of vegetations, details about valve function, or type or severity of degenerative structural heart diseases was available. This would have been interesting to present together with the results, as these factors can contribute to important aspects of bacterial aetiology, manifestations and mortality. Information about the time for primary prosthetic valve implantation was not available in the SRIE, which made it impossible to present or distinguish between early- and late-onset prosthesis-related IE. Further, no blood test results or follow-up information such as 1-year mortality was included in the SRIE. Unfortunately, it was not possible to analyse more unusual aetiologies of IE, such as gram-negative bacteria and fungi, because there were very few cases with these pathogens in our study. Future studies with larger study populations and prospective designs would be of interest to explore the possible associations between manifestations detected by ECHO, and bacterial aetiology in IE. It should

be noted that **Paper IV** reflects a population of patients with IE, admitted to a university TH in Stockholm, Sweden, so the results might differ from other populations of patients with IE. In this thesis, possible benefits of implementing ECHO in resource-limited settings have been discussed. However, the equipment used at the KUH might not be comparable to the equipment that would possibly be used in resource-limited settings. The equipment used in such settings could be less advanced, and the ability to identify IE manifestations with such equipment would need to be evaluated before implementation.

The SRIE includes information on antibiotic prescribing, which generally followed the Swedish national guidelines for antibiotic treatment of IE. I considered including the antibiotic prescribing among the patients with IE in **Paper IV**, but chose not to, as the purpose of the paper was to include the diagnostics aspect rather than the antibiotics aspect.

6 CONCLUSIONS

The main conclusions from this thesis are as follows.

- Antibiotics were commonly prescribed at the two study hospitals to patients with no evident indication for such treatments, indicating less rational use of antibiotics.
- Adherence to recommendations regarding antibiotic treatment for given indications varied between the hospitals, suggesting there is a need for improved knowledge about guidelines and lists of essential medicines among the prescribers.
- Prescribing FDCs of antibiotics was higher at the NTH than at the TH. Prescribing FDCs of antibiotics increased over 2008–2017 at both hospitals.
- Extensive prescribing of broad-spectrum antibiotics was seen at both hospitals, although diagnostic methods were rarely used to confirm or exclude infections or their bacterial aetiologies.
- ECHO as part of the standard management of IE in high-income settings contributes to verification of the diagnosis, evaluation and follow-up.
- ECHO can be used to identify cardiac manifestations of IE but might also give a suggestion of bacterial aetiology as some IE manifestations detected by ECHO have been shown to be associated with certain bacterial species.

7 REFLECTIONS FOR THE FUTURE

7.1 RESEARCH

This thesis presents different ways of analysing data regarding antibiotic prescribing practices in a resource-limited setting. It further presents an evaluation of ECHO as a diagnostic method for IE in a high-income setting, suggesting that assessment of cardiac manifestations by ECHO can help evaluate the severity of the disease and might also indicate the bacterial aetiology in patients with IE. These methods of analysing data can be reused for future investigations. The findings from the studies in this thesis suggest some future research questions as follows.

- Why are antibiotics prescribed to patients with no apparently proper indication for antibiotic treatment?
- How can antibiotic prescribing guidelines or essential lists of medicines be implemented in resource-limited settings to improve prescribing patterns?
- Why are diagnostic methods not used among prescribers with access to such methods in resource-limited settings?
- Do interventions that target physicians' knowledge, attitude and practice towards antibiotic prescribing and the management of infectious diseases affect their practice?
- Would implementation of diagnostic methods improve the management of infectious diseases and rationalize the antibiotic prescribing practices in resource-limited settings?
- Would implementation of ECHO in a resource-limited setting improve the management of IE?
- Would it be possible to find associations between bacterial species and IE manifestations detected by ECHO in samples of patients with IE from populations in other settings, for instance in resource-limited settings? If so, do the associations differ between different settings?

7.2 POLICY AND PRACTICE

There is a global need for the improved use of antibiotics and management of infectious diseases (9, 10, 12, 162, 163). Interventions should be designed to ensure the long-term successful prevention and treatment of infectious diseases, with the intention of keeping antibiotics as an available, effective and safe treatment with quality-assured medicines (10). These interventions should be implemented at all levels of health care as well as in the general public (8). Interventions need to be adapted to the setting in which they are aimed to be implemented, to increase their impact (19). Interventions improving the use of antibiotics and management of infectious diseases in resource-limited settings – such as the implementation of an antibiotic stewardship programme and enforcement of an antibiotic restriction policy at ICUs–have shown promising results (112, 164). The results presented in this thesis suggest further interventions to improve antibiotic prescribing practices and management of infectious diseases in hospitals in the Ujjain district as well as in other hospitals in similar resource-limited settings. Obstacles and contributing factors, suggested improvements and goals for rationalizing the antibiotic prescribing and improve the management of infectious diseases in the NTH and the TH as well as similar resource-limited settings, are presented in Figure 5.

Further, lack of diagnostics complicates the management of patients with suspected infectious diseases, both in terms of determining the site of infection and the infectious etiology. The results from this thesis show that ECHO is a method that can be used to diagnose IE, to provide additional information about possible cardiac comorbidity, and to evaluate the severity of the disease. This is valuable information that can guide the management of the patients with IE. By implementing ECHO in a resource-limited setting, the management of the patients with IE could possibly be improved.

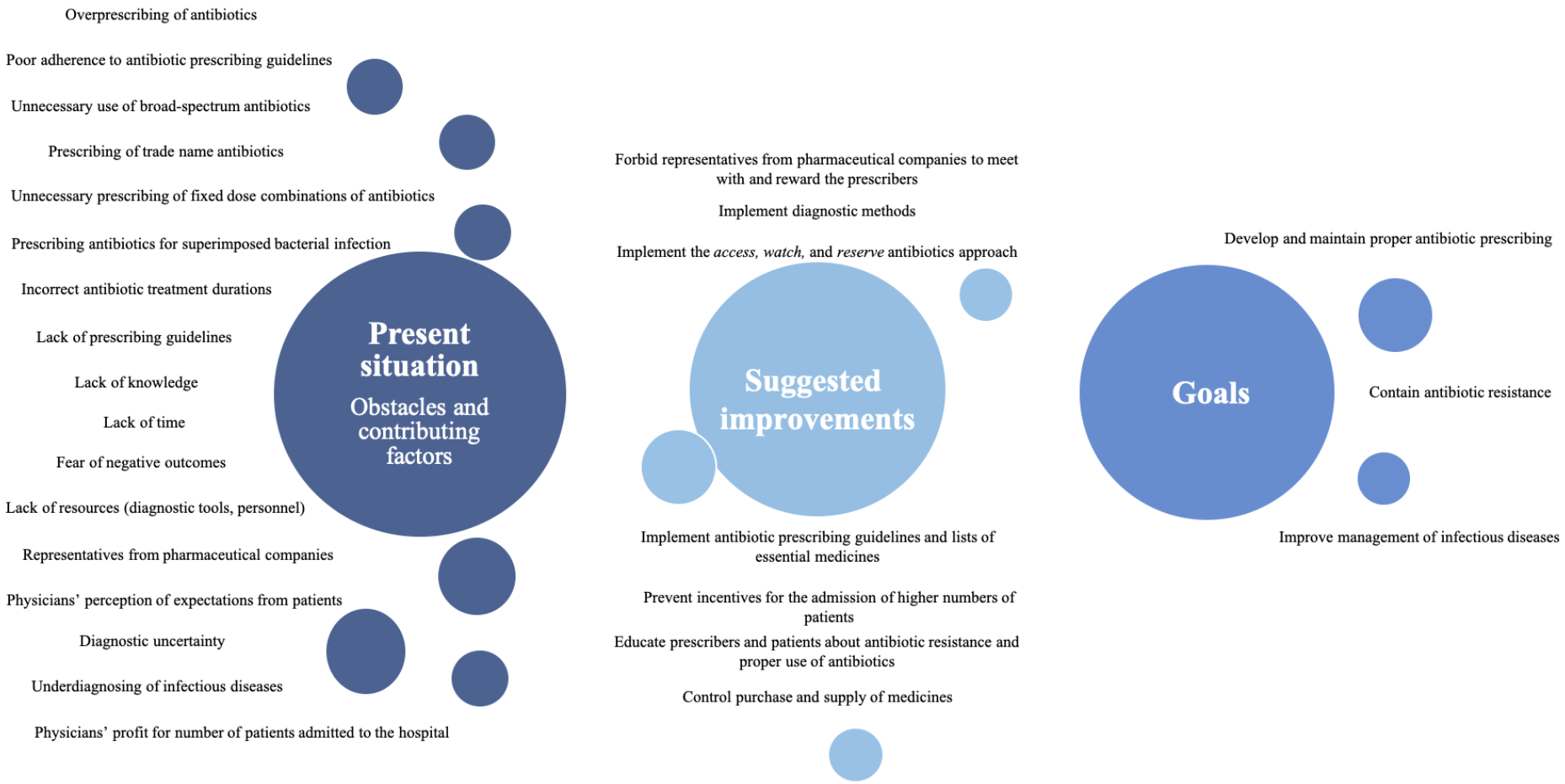

Figure 5. Present situation, suggested improvements and goals for improvement of antibiotic prescribing to contain antibiotic resistance in resource-limited settings.

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APPENDIX I. ANTIBIOTICS GROUPED BY THEIR ANATOMICAL THERAPEUTIC CHEMICAL CLASSIFICATION CODE AND CATEGORIZED AS *ACCESS*, *WATCH*, *RESERVE* ANTIBIOTICS OR FIXED-DOSE COMBINATIONS OF ANTIBIOTICS (PAPER III)

ATC code	Antibiotic group	Specific antibiotics	Antibiotic category
J01A	Tetracyclines	Doxycycline	*Access*
		Tigecycline	*Reserve*
J01B	Amphenicols	Chloramphenicol	*Access*
J01 group 1: J01CA, J01CE, J01CF, J01CG	Penicillins with extended spectrum, β-lactamase sensitive penicillins, β-lactamase resistant penicillins, β-lactamase inhibitors	Amoxicillin, ampicillin, benzathine benzylpenicillin, benzylpenicillin, cloxacillin, phenoxymethylpenicillin, procaine benzylpenicillin, piperacillin, tazobactam	*Access*
J01CR	Combinations of penicillins including β-lactamase inhibitors	Amoxicillin with clavulanic acid	*Access*
		Piperacillin with tazobactam	*Watch*
J01D	β-lactam antibiotics	Cefalexin, cefazolin, cefadroxile, cefradine, cefuroxime	*Access*
		Cefixime, ceftriaxone, cefotaxime, cefoperazone, cefodoxime, ceftazidime, meropenem, imipenem, cilastin, faropenem	*Watch*
		Aztreonam, cefepime, ceftaroline	*Reserve*
J01E	Sulphonamides and trimethoprim	Sulfamethoxazole with trimethoprim	*Access*
J01F	Macrolides	Clindamycin	*Access*
		Azithromycin, clarithromycin, erythromycin, lincomycin, roxithromycin	*Watch*
J01G	Aminoglycosides	Gentamicin, netilmicin, kanamycin, tobramycin, streptomycin, amikacin	*Access*
J01M	Quinolones and fluoroquinolones	Ciprofloxacin, levofloxacin, moxifloxacin, norfloxacin, orfloxacin, gemifloxacin, pazufloxacin, gatifloxacin, prulifloxacin	*Watch*

ATC code	Antibiotic group	Specific antibiotics	Antibiotic category
J01R	Combinations of antibiotics	Ampicillin with cloxacillin; amoxicillin with cloxacillin; azithromycin with ambroxol; cefixime with ornidazole; cefoperazone with sulbactam; ceftriaxone with sulbactam; ceftriaxone with tazobactam; norfloxacin with tinidazole; ofloxacin with ornidazole; ofloxacin with tinidazole; cefixime with clavulanate potassium; cefixime with clavulanic acid; cefixime with cloxacilline; cefixime with ofloxacin; cefixime with tazobactam; cefotaxime with sulbactam; cefpodoxime with clavulanic acid; cefpodoxime with cloxacillin; cefpodoxime with dicloxacillin; meropenem with sulbactam; ceftazidime with tazobactam; cefuroxime with clavulanic acid; ciprofloxacin with ornidazole; ciprofloxacin with tinidazole; efoperazone with sulbactam; levofloxacin with ornidazole; cefixime with azithromycin; cefpodoxime with potassium clavulanate; ceftriaxone with clavulanic acid	FDCs of antibiotics
J01X	Other antibiotics	Metronidazole (J01XD01), nitrofurantoin, tinidazole, ornidazole, spectinomycin	*Access* (P01AB01 Metronidazole included)
		Teicoplanin, vancomycin	*Watch*
		Polymyxin B, colistin, fosfomycin, linezolid, daptomycin	*Reserve*

For antibiotic classification, the 2019 ATC classification was used. ATC, Anatomical Therapeutic Chemical; FDC, fixed-dose combination.

APPENDIX II. ANTIBIOTICS PRESCRIBED FOR PATIENTS WITH SEVERE INFECTIONS AT THE NTH AND TH IN 2008–2017 (PAPER III)

Antibiotic prescribing in DDDs per 1000 patient days

Cellulitis at the NTH	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**	**2017**	**%**	**Slope, t[a]**	**P value[b]**
J01CR	4.12	4.44	0.73	4.78	3.17	4.90	5.77	4.33	3.24	7.11	16	2.32	**0.02**
J01D	13.11	9.01	1.98	11.52	5.09	4.28	12.50	7.54	14.18	3.06	32	6.33	**<0.01**
J01R	6.53	5.60	3.12	2.60	4.70	3.46	3.01	6.78	6.12	1.84	17	6.97	**<0.01**
J01X	9.25	15.76	3.68	3.34	1.30	1.05	1.62	1.08	1.42	1.48	15	3.31	**<0.01**
Cellulitis at the TH	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**	**2017**	**%**	**Slope, t[a]**	**P value[b]**
J01CR	10.84	7.45	4.24	7.14	9.47	8.99	10.07	8.03	16.29	13.33	19	4.91	**<0.01**
J01D	4.48	6.01	9.32	6.22	4.76	4.66	6.87	6.53	9.98	2.10	12	5.58	**<0.01**
J01G	9.33	13.53	9.11	9.26	14.55	10.47	10.01	6.37	15.64	5.45	21	0.44	0.66
J01M	10.20	8.70	3.35	5.66	9.00	4.51	4.30	3.31	9.11	6.22	13	2.66	**<0.01**
J01X	6.53	3.70	7.38	6.50	6.86	10.22	7.89	3.84	9.17	3.91	13	2.27	**0.02**

Peritonitis at the NTH	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	%	Slope, t[a]	P value[b]
J01CR	3.71	4.26	3.85	10.23	3.06	5.70	4.45	5.89	5.67	4.40	16	4.98	**<0.01**
J01D	26.28	6.43	4.54	4.34	10.36	7.35	1.41	7.78	8.04	5.06	25	1.34	0.18
J01R	8.58	6.14	5.29	5.55	5.01	1.14	3.09	5.02	6.19	1.40	15	8.67	**<0.01**
J01X	16.03	11.72	11.64	9.09	4.57	2.49	1.58	1.81	3.54	1.97	20	10.95	**<0.01**
Peritonitis at the TH	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**	**2017**	**%**	**Slope, t[a]**	**P value[b]**
J01CR	5.84	4.39	6.68	5.55	10.92	9.13	16.54	13.44	13.36	5.35	15	10.16	**<0.01**
J01D	4.00	4.95	9.63	9.44	6.87	2.76	6.04	7.64	15.87	7.73	12	17.55	**<0.01**
J01G	10.08	4.65	11.08	9.94	11.84	6.49	11.43	11.87	8.64	4.08	15	8.13	**<0.01**
J01M	16.05	10.72	3.27	6.40	11.99	5.83	11.60	12.86	15.42	3.56	16	2.92	**<0.01**
J01X	15.75	9.55	16.85	17.73	15.44	13.92	22.64	17.30	23.05	5.24	26	8.69	**<0.01**

Pneumonia at the NTH	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	%	Slope, t[a]	P value[b]
J01CR	11.54	5.01	4.05	4.27	4.95	4.61	5.96	5.79	8.79	8.17	29	-0.91	0.36
J01D	7.75	6.92	4.22	8.53	3.24	2.95	1.37	4.44	4.23	4.18	22	-1.38	0.17
J01M	1.00	0.49	0.47	0.50	2.41	0.83	3.98	7.34	3.47	1.18	10	2.54	**0.01**
J01R	5.74	4.61	3.51	2.55	4.21	4.76	5.27	4.61	3.72	4.82	20	5.65	**<0.01**

Pneumonia at the TH	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	%	Slope, t[a]	P value[b]
J01A	16.44	11.85	14.23	8.45	13.76	10.18	7.21	2.91	2.25	18.79	25	1.81	0.07
J01CR	8.45	12.64	10.66	1.91	8.26	15.71	20.02	19.21	15.38	13.27	30	6.65	**<0.01**
J01D	3.85	6.01	5.04	10.91	7.24	4.65	3.94	3.81	15.31	20.74	19	12.87	**<0.01**
J01M	15.87	9.36	8.72	5.72	3.88	2.04	1.61	1.77	4.65	7.44	14	1.89	0.06

Sepsis at the NTH	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	%	Slope, t[a]	P value[b]
J01CR	5.05	3.90	2.13	1.97	2.23	2.55	3.01	6.90	3.47	6.66	21	1.10	0.27
J01D	10.74	7.47	1.19	3.27	4.63	3.86	4.77	6.37	4.29	1.70	27	-4.67	**<0.01**
J01R	7.55	3.66	1.71	1.33	2.09	3.36	1.84	3.07	2.63	2.76	17	3.17	**<0.01**
J01X	12.22	6.54	4.51	0.99	0.79	0.00	1.28	1.02	0.72	0.00	16	-6.07	**<0.01**
Sepsis at the TH	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**	**2017**	**%**	**Slope, t[a]**	**P value[b]**
J01A	14.61	7.67	9.59	1.83	4.31	2.95	4.26	0.00	0.00	3.13	11	-5.50	**<0.01**
J01CR	1.84	11.18	27.87	2.76	5.09	1.83	8.04	7.09	7.79	7.09	19	-5.07	**<0.01**
J01D	1.37	5.48	8.48	3.03	5.87	3.79	12.27	5.57	7.21	4.89	13	-5.20	**<0.01**
J01M	11.51	1.75	14.45	11.32	3.13	0.11	4.75	0.91	2.81	3.62	13	-8.49	**<0.01**
J01X	8.95	12.93	15.34	7.08	9.18	6.36	12.60	0.91	6.00	4.07	19	-15.48	**<0.01**

The antibiotics presented comprised ≥75% of the total amount of antibiotic prescribing within each diagnosis group. Numbers are presented as total incidences of antibiotic prescribing of antibiotic groups for each year, in DDD/1000 patient days and as percentage of the total prescriptions of antibiotics for each diagnosis and hospital during the study period. [a] t values were obtained by linear regression; a positive t indicates a positive trend and a negative t indicates a negative trend of prescribing. [b]P values were obtained from linear regression, evaluating the differences in antibiotic prescribing over the 10-year period. Statistically significant P values indicate a significant trend and are marked in bold font. DDD, defined daily doses; NTH, non-teaching hospital; TH, teaching hospital.

APPENDIX III. DISTRIBUTION OF IE MANIFESTATIONS AND BACTERIAL SPECIES COMPRISING 90% OF THE AETIOLOGIES AMONG PATIENTS WITH IE (PAPER IV)

	Aortic valve vegetation	Mitral valve vegetation	Tricuspid valve vegetation	Pulmonary valve vegetation	CIED-associated IE	Perivalvular abscess	Total, n (%)
All patients, n (%)	190 (39)	195 (40)	108 (22)	9 (2)	24 (5)	29 (6)	
Staphylococcus aureus	**68 (14), 0.43, 0.29-0.63; <0.01**	84 (17), 0.69, 0.47-1.01; 0.05	**88 (18), 6.79, 3.93-12.11; <0.01**	**8 (2), 8.73, 1.15-388.69; 0.01**	11 (2), 0.89, 0.35-2.20; 0.78	11 (2), 0.63, 0.26-1.44; 0.24	239 (49)
CoNS	**16 (3), 3.38,1.33-9.29; <0.01**	6 (1), 0.49, 0.16-1.33; 0.13	2 (0), 0.31, 0.03-1.30; 0.13	0 (0)	**4 (1), 4.48, 1.01-15.15; 0.02**	**5 (1), 4.87, 1.30-15.01; <0.01**	24 (5)
Viridans group streptococci	45 (9), 1.33, 0.84-2.12; 0.20	**51 (10), 1.70, 1.07-2.71; 0.02**	**5 (1), 0.14, 0.04-0.36; <0.01**	0 (0)	1 (0), 0.16, 0.04-1.00; 0.04	6 (1), 1.00, 0.32-2.61; 1.00	102 (21)
Group B streptococci	4 (1), 0.91, 0.19-3.62; 1.00	**9 (2), 7.13, 1.44-68.33;<0.01**	0 (0)	0 (0)	0 (0)	1 (0), 1.62, 0.04-12.11; 0.49	11 (2)
Group D streptococci	3 (1), 1.20, 0.17-7.15; 1.00	4 (1), 2.05, 0.34-14.14; 0.44	0 (0)	0 (0)	0 (0)	1 (0), 2.70, 0.06-23.37; 0.35	7 (1)
Group G streptococci	4 (1), 1.60, 0.29-8.70; 0.49	3 (1), 0.91, 0.14-4.73; 1.00	1 (0), 0.50, 0.01-3.99; 1.00	0 (0)	0 (0)	0 (0)	8 (2)
Enterococcus faecalis	**30 (6), 2.64, 1.40-5.08; <0.01**	19 (4), 0.93, 0.48-1.75; 0.80	7 (1), 0.55, 0.20-1.28; 0.15	0 (0)	2 (0), 0.80, 0.09-3.41; 1.00	2 (0), 0.64, 0.07-2.69; 0.76	50 (10)
HACEK	6 (1), 1.06, 0.31-3.40; 0.91	3 (1), 0.37, 0.07-1.40; 0.18	2 (0), 0.54, 0.06-2.44; 0.54	1 (0), 4.19, 0.09-34.97; 0.25	**4 (1), 8.31, 1.76-31.08;<0.01**	1 (0), 1.15, 0.03-8.07; 0.60	15 (3)

Values are presented as n,number of patients; (% of patients); odds ratio; 95% confidence interval; P values. Statistically significant associations (P < 0.05) are shown in bold. CIED, cardiovascular implantable electronic device; CoNS, coagulase-negative staphylococci; HACEK, combination of *Haemophilus* species, *Aggregatibacter* species, *Cardiobacterium hominis*, *Eikenella corrodens*, and *Kingella kingae;* IE, infective endocarditis; KUH, Karolinska University Hospital.

APPENDIX IV. DISTRIBUTION OF BACTERIAL AETIOLOGIES, PREDISPOSING FACTORS AND OUTCOMES AMONG PATIENTS WITH IE AT THE KUH (PAPER IV)

	Prosthetic heart valve	History of IV drug abuse	>1 ECHO manifestation	Surgical treatment for IE	In-hospital mortality
All patients, n (%)	92 (19)	156 (32)	83 (17)	139 (28)	33 (7)
Staphylococcus aureus	**28 (30), 0.39, 0.23-0.65; <0.01**	**122 (78), 6.72, 4.24-10.76; <0.01**	36 (43), 0.78, 0.47-1.28; 0.30	61 (44), 0.77, 0.51-1.16; 0.19	21 (64), 1.93, 0.88-4.42; 0.07
CoNS	8 (9), 2.29, 0.82-5.88; 0.06	**2 (1), 0.19, 0.02-0.77; 0.01**	**11 (13), 4.65, 1.80-11.71; <0.01**	10 (7), 1.88, 0.73-4.67; 0.13	2 (6), 1.28, 0.14-5.62; 0.67
Viridans group streptococci	14 (15), 0.64, 0.32-1.20; 0.15	**8 (5), 0.14, 0.06-0.30; <0.01**	14 (17), 0.74, 0.37-1.41; 0.34	34 (24), 1.36, 0.82-2.22; 0.20	6 (18), 0.84, 0.28-2.16; 0.71
Group B streptococci	1 (1), 0.43, 0.01-3.09; 0.70	0 (0)	2 (2), 1.10, 0.11-5.44; 1.00	3 (2), 0.95, 0.16-4.04; 1.00	1 (3), 1.38, 0.04-10.22;0.54
Group D streptococci	3 (3), 3.34, 0.48-20.04; 0.13	0 (0)	1 (1), 0.82, 0.02-6.89; 1.00	4 (3), 3.46, 0.57-23.84; 0.10	0 (0)
Group G streptococci	2 (2), 1.46, 0.14-8.33; 0.65	1 (1), 0.30, 0.01-2.40; 0.45	0 (0)	1 (1), 0.36, 0.01-2.83; 0.45	0 (0)
Enterococcus faecalis	**17 (18), 2.52, 1.25-4.93; <0.01**	17 (11), 1.12, 0.57-2.16; 0.71	11 (13), 1.45, 0.64-3.05; 0.31	9 (6), 0.53, 0.22-1.14; 0.09	2 (6), 0.55, 0.06-2.29; 0.56
HACEK	5 (5), 2.24, 0.58-7.39; 0.17	0 (0)	4 (5), 1.83, 0.41-6.38; 0.30	**8 (6), 3.02, 0.93-9.96; 0.03**	1 (3), 0.99, 0.03-6.94; 1.00

Values are presented as n, number of patients; (% of patients); odds ratio; 95% confidence interval; P values. Statistically significant associations ($P < 0.05$) are shown in bold. CoNS, coagulase-negative staphylococci; ECHO, echocardiography; HACEK, combination of *Haemophilus* species, *Aggregatibacter* species, *Cardiobacterium hominis*, *Eikenella corrodens* and *Kingella kingae*; IE, infective endocarditis; IV, intravenous; KUH, Karolinska University Hospital.

APPENDIX V. DISTRIBUTION OF ECHO MANIFESTATIONS, PREDISPOSING FACTORS AND OUTCOMES AMONG PATIENTS WITH IE AT THE KUH (PAPER IV)

	History of IV drug abuse	Surgical treatment for IE	In-hospital mortality
All patients, n (%)	156 (32)	139 (28)	33 (7)
Aortic valve vegetation	**31 (20), 0.28, 0.17-0.44; <0.01**	**72 (52), 2.14, 1.41-3.25; <0.01**	17 (52), 1.76, 0.81-3.82; 0.12
Mitral valve vegetation	**44 (28), 0.48, 0.31-0.74; <0.01**	64 (46), 1.45, 0.95-2.19; 0.07	15 (45), 1.29, 0.59-2.79; 0.48
Tricuspid valve vegetation	**86 (55), 17.54, 9.99-31.33; <0.01**	**19 (14), 0.47, 0.25-0.82; 0.01**	3 (9), 0.34, 0.06-1.12; 0.08
Pulmonary valve vegetation	**7 (4), 7.85, 1.46-77.92; <0.01**	1 (1), 0.31, 0.01-2.37; 0.46	0 (0)
CIED-associated IE	**1 (1), 0.09, 0.01-0.55; <0.01**	**18 (13), 8.60, 3.16-26.96; <0.01**	2 (6), 1.28, 0.14-5.61; 0.67
Perivalvular abscess	**4 (3), 0.33, 0.08-0.97; 0.04**	**17 (12), 3.96, 1.72-9.34; <0.01**	**6 (18), 4.21, 1.29-11.81; <0.01**
Prosthetic heart valve	**12 (8), 0.28, 0.13-0.53; <0.01**	26 (19), 1.00, 0.58-1.69; 1.00	8 (24), 1.43, 0.54-3.41; 0.40

Values are presented as n, number of patients; (% of patients); odds ratio; 95% confidence interval; and P values. Statistically significant associations ($P < 0.05$) are shown in bold. CIED, cardiovascular implantable electronic device; IE, infective endocarditis; IV, intravenous; KUH - Karolinska University Hospital.